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556. THE USE OF SEAWEED MEALS IN CONCENTRATE MIXTURES FOR DAIRY COWS

BY A. W. A. BURT, S. BARTLETT AND S. J. ROWLAND
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There has recently been some interest in the possibility of increasing the utilization of seaweed for livestock feeding. Only the brown seaweeds occur in sufficient quantity to justify their collection for this purpose. Quantitative surveys of the main deposits around the Scottish coasts(1) showed deposits of over 180,000 tons of the littoral (mainly *Ascophyllum nodosum*) and about 10,000,000 tons of the sublittoral (mainly *Laminaria cloustoni*) brown seaweeds.

Black(2) and Woodward(1) reviewed various experiments on the use, chiefly in the rations of sheep, pigs and poultry, of seaweed meals for stock food, and concluded that ruminants make better use of seaweed meals than do pigs. Senior, Collins & Kelly(3) in digestibility trials with sheep, found that their best seaweed sample, *Laminaria* collected in the autumn, had a feeding value comparable to that of meadow hay. The same workers observed that the protein in these materials generally had a low and sometimes a negative digestibility. This was confirmed in experiments at the Rowett Research Institute (unpublished; quoted by Black(2) and Woodward(1)), and *Laminaria* meal was found to be more digestible than *Ascophyllum*.

Very little critical work has been done on the value of seaweed meals for dairy cows, although these materials are being used commercially. Dunlop(4), using cows on seventeen farms, studied the effect of substituting approximately 200 g. of *Ascophyllum* meal daily for a similar amount of the normal concentrate ration, and concluded that there were considerable increases in butterfat production.

In view of the lack of information, obtained under carefully controlled conditions, about the value of seaweed meals to dairy cows, it was decided to compare samples of the two main types of seaweed meal with a feed of known nutritive and commercial value. An experiment has therefore been carried out to compare the effects on the yield and composition of milk, of concentrate mixtures containing (*a*) *A. nodosum* meal, (*b*) *Laminaria cloustoni* meal, (*c*) oatfeed and common salt. Black(2) and Dunlop(5) indicated that seaweed meals were relatively unpalatable when fed to dairy cows, and this was confirmed in preliminary small-scale trials in the Institute herd. It was evident that not more than about 10% of seaweed meal could be used, and that this would have to form part of a concentrate mixture containing ingredients of high palatability.

MATERIAL AND METHODS

The *Ascophyllum nodosum* used was collected in the spring of 1953 and the *Laminaria cloustoni* in the autumn of 1952: both were dried and milled to produce suitable meals. The oatfeed used was the by-product of the production of oatmeal and had a calculated starch equivalent of 45.

Analyses of the two seaweed meals and the oatfeed are given in Table 1. The composition of the former agrees with data quoted by Black (2), after making due allowance for the considerable variation which occurs naturally in these materials. The oatfeed differed from the seaweed mainly in having a higher percentage of fibre and soluble carbohydrate, and a lower ash content.

Table 1. *Chemical composition of the seaweed meals and oatfeed*

(Percentage of dry matter.)

Food	Dry matter (%)	Crude protein	True protein	Ether extract	Fibre	Nitrogen-free extractives	Ash	CaO	P_2O_5	NaCl
Ascophyllum nodosum meal	88·56	6·93	5·69	3·33	5·49	60·82	23·43	2·52	0·262	3·82
Laminaria cloustoni meal	90·83	11·98	8·32	0·66	8·70	52·75	25·91	2·63	0·590	11·91
Oatfeed	91·00	8·48	7·81	4·79	16·59	67·33	2·81	0·13	0·729	0·20

Three concentrate mixtures, designated A, B and C, containing *Ascophyllum nodosum* meal, *Laminaria cloustoni* meal and oatfeed respectively were prepared and cubed. The constituents of these mixtures and their relative proportions are shown in Table 2. In the control mixture C, $8\frac{3}{4}$% of oatfeed and $1\frac{1}{4}$% common salt were used, in contrast to 10% of seaweed meal in the other mixtures, the salt being added in order to make some adjustment for the much lower salt content, and higher soluble carbohydrate content of the oatfeed. The chemical composition of the three concentrate mixtures in Table 3 shows very little variation between them.

Table 2. *Composition of the concentrate mixtures, A, B and C*

(Percentage by weight.)

Mixture A 10% *Ascophyllum nodosum* meal
Mixture B 10% *Laminaria cloustoni* meal
Mixture C $8\frac{3}{4}$% oatfeed plus $1\frac{1}{4}$% common salt
Each with 90% of the following:

15 barley meal	10 decort. cotton cake
$31\frac{1}{4}$ maize germ meal	15 decort. groundnut cake
5 rice meal	$2\frac{1}{2}$ dicalcium phosphate
5 molasses	$1\frac{1}{4}$ limestone flour
5 linseed cake	

Table 3. *Chemical composition of the foods*

(Percentage of samples as fed.)

Food	Crude protein	True protein	Ether extract	Fibre	Nitrogen-free extractives	Moisture	Ash	CaO	P_2O_5	NaCl
Concentrates:										
Mixture A	17·74	16·71	6·68	4·82	50·22	11·82	8·72	2·22	2·33	0·62
Mixture B	17·88	16·22	6·20	5·22	49·69	12·22	8·79	2·01	2·29	1·26
Mixture C	17·12	16·86	6·75	5·37	50·96	12·38	7·42	1·60	2·20	1·34
Hay*	7·89	7·02	1·56	27·02	36·78	21·36	5·39	—	—	—
Kale*	1·68	1·25	0·31	2·87	9·03	84·7	1·41	—	—	—

* Mean values for three samples.

Eighteen high-yielding Ayrshire cows were used in this experiment, twelve of which were in the first 2 months of lactation, and six in the third and fourth months, when the experiment started.

The layout of the trial was similar to that described by Cochran, Autrey & Cannon (6), and consisted of two complementary 3 × 3 Latin squares as follows, replicated three times:

	Cow			Cow		
Period	1	2	3	4	5	6
I	A	B	C	A	B	C
II	B	C	A	C	A	B
III	C	A	B	B	C	A

where A, B and C are the three concentrate mixtures.

An initial control period of 2 weeks on normal herd rations was followed by three experimental periods, each of 3 weeks' duration. Owing to the relatively small differences between the concentrate mixtures, the changeover from one type to another was made abruptly.

A basal roughage ration consisting of 30 lb. marrowstem kale and an average of 18 lb. hay, estimated to supply nutrients for maintenance and the production of the first 1½ gal. of milk, was fed daily to each cow throughout. The amount of hay fed to each cow was based on her live weight in the control period as estimated by taking chest girth measurements and using the conversion tables for the Ayrshire breed given by Davis, Morgan, Brody & Ragsdale (7). A difference in estimated live weight of 70 lb. from the standard of 1000 lb. was met by an adjustment of 1 lb. hay/day to the average quantity of 18 lb.

Concentrate cubes A, B, or C were fed at the rate of 3½ lb./gal. of milk above the first 1½ gal. The quantity of concentrates was originally based on the yield of each cow during the control period, and was subsequently adjusted by a reduction of 1 lb./head/day at each changeover.

The cows were fed individually, and all food offered, and refusals were weighed. Milking was twice daily and yields were recorded at each milking. Samples for the determination of the fat and solids-not-fat percentages of the milk were taken at four consecutive milkings in each week, and a single weighted composite sample prepared for each cow for analysis. The foods were analysed by the conventional methods: the fat content of the milk was determined by the Gerber method, and the total solids content gravimetrically.

At the end of the control period and each experimental period, the chest girth of each cow was recorded and its fatness and the handling properties of the skin were estimated subjectively on scales ranging from 0 to 10 points.

RESULTS

The mean milk yield and percentages of fat and solids-not-fat for each treatment, computed from the data for the last week of each experimental period, are shown in Table 4. The solids-not-fat percentages were expressed in terms of the fat-free milk before computation. One cow had a severe attack of mastitis in the last experimental period and her values for this period were calculated using the missing plot technique applied by Blaxter & French (8) to experiments of this type.

The differences between the mean milk yields produced under each treatment were negligible. The lowest mean fat percentage was associated with mixture B and the highest with mixture A. Analysis of variance showed that differences between treatments in

Table 4. *Mean milk yield, fat, and solids-not-fat percentages for each treatment computed from the values for the last week of each feeding period*

Treatment	Mean milk yield (lb./cow/week)	Mean fat (%)	Mean solids-not-fat (%)
Concentrate A	312·6	3·68	8·70
Concentrate B	309·7	3·52	8·65
Concentrate C	309·9	3·60	8·79

milk yield and fat percentage were not statistically significant. The milk yield analysis gave a value for the standard error of a treatment mean weekly yield/cow of ±3·43 lb., or 1·1 % of the general mean. On the basis of this low standard error, if a difference between treatments of 10·5 lb./cow/week had been observed it would have been regarded as statistically significant.

The analysis of variance for solids-not-fat is shown in Table 5. There was a significant group-treatment interaction indicating that the effect of treatment varied from group to group, and a significant variation between treatments. The means and standard error

Table 5. *Analysis of variance of solids-not-fat percentages*

	D.F.	S.S.	M.S.	Significance of *F*
Total	53	5·3194	—	—
Between groups	5	0·7321	0·1464	**
Cows within groups	12	3·4814	0·2901	**
Periods within groups	12	0·6284	0·0524	**
Treatments	2	0·1544	0·0772	**
Treatment × group	10	0·2547	0·0255	**
Error	12	0·0684	0·0057	—

** Significant at the 0·01 level of probability.

	Treatment means	S.E.M. of 18 cows
A	8·70	±0·0178
B	8·65	
C	8·79	

quoted at the bottom of Table 5 show that the mean solids-not-fat percentage for mixture C was significantly greater than for mixtures A and B, and that there was no significant difference between A and B.

No significant effects of treatment upon fatness, skin-handling properties, or chest girth were observed.

The amounts of food offered to, and refused by, the cows on each treatment are shown in Table 6. The only appreciable refusal of concentrates was by one cow receiving mixture B in the last experimental period. She then refused an average of 2·4 lb. out of a daily allowance of 10¼ lb. of concentrates. Calculation showed that the cows received very slightly less starch equivalent and digestible protein than their requirements for maintenance and production according to the standards recommended by Woodman (9).

Table 6. *Total amounts of each food offered and refused*

	Offered (lb.)			Refused (lb.)		
Treatment	Concentrates	Hay	Kale	Concentrates	Hay	Kale
Concentrate A	4968	6951	11,340	—	191	401
Concentrate B	4968	6951	11,340	75	149	279
Concentrate C	4968	6951	11,340	—	186	383

DISCUSSION

The results of this experiment showed no significant differences in their effects upon milk yield between concentrates containing 10% of seaweed meals or 8¾% oatfeed and 1¼% salt. Although the treatment differences studied were relatively slight, the technique used was capable of measuring small effects upon yield as shown by the very low error in the analysis of variance. It is noteworthy that high-yielding cows, producing an average of 45·3 lb. milk/head/day over the whole experiment, were used, and the feeding records show that they were not overfed.

No significant differences in fat percentage occurred between the treatments, in contrast to the conclusions of Dunlop (4), in spite of feeding amounts of seaweed meal which averaged 1·3 lb./cow/day, a quantity considerably greater than was used by him.

It is difficult to account for the significant treatment effects upon solids-not-fat percentage. The significant interaction in the analysis of variance indicates that the effect was not consistent from group to group. Reductions in the intake of energy or protein lower solids-not-fat percentage (10), and it may be that the small differences observed in this experiment reflected a slightly lower availability of nutrients from the concentrates containing the seaweed meals.

This experiment indicates that, in practice, the nutritive value of the seaweed meals is no greater than that of a mixture of 7 parts oatfeed and 1 part common salt. In appraising the value of the seaweed meals in this way it should be remembered that the oatfeed-salt mixture had a starch equivalent of only 39—a low value for an ingredient of concentrate mixtures for dairy cows. This result is in accord with the relatively low nutritive value previously ascribed to seaweed meals by Senior *et al.* (3), and with the findings at the Rowett Research Institute (1,2).

Claims are sometimes made for beneficial effects upon the dairy cow, arising from the vitamin and high mineral content of seaweed meals, but supporting evidence is lacking.

In view of the cost of collection, the need for artificial drying, the low palatibility and relatively low nutritive value of seaweed meals, the practical value of these materials in the feeding of dairy cows appears to be very limited at the present time.

SUMMARY

The relative effects upon milk yield and composition of three concentrate mixtures containing 10% of two types of seaweed meal (*Ascophyllum nodosum* and *Laminaria cloustoni*) and an oatfeed-salt mixture, have been observed.

Each cow received daily a basal ration of 30 lb. marrowstem kale and 18 lb. hay for maintenance and the first 1½ gal. of milk and 3½ lb. concentrates for each gallon thereafter.

No significant effects of treatment upon milk yield or fat percentage were observed.

The milk produced on the oatfeed-salt mixture had a slightly but significantly higher solids-not-fat content than that on the mixtures containing seaweed meals.

The results showed that seaweed meals have a nutritive value no greater than that of a mixture of 7 parts oatfeed and 1 part salt, which had an estimated starch equivalent of only 39.

In view of the relatively high cost, low palatability and low nutritive value of seaweed meals, their possible use in the feeding of dairy cows appears very limited.

We should like to thank Mr W. D. Hay, O.B.E., B.Sc., Director of the Ministry of Agriculture and Fisheries Bridget's Experimental Husbandry Farm, Martyr Worthy, nr. Winchester, Hants, for his willing co-operation in providing facilities, and the farm staff for their help in carrying out this experiment.

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557. STUDIES OF THE SECRETION OF MILK OF LOW FAT CONTENT BY COWS ON DIETS LOW IN HAY AND HIGH IN CONCENTRATES

IV. THE EFFECT OF VARIATIONS IN THE INTAKE OF DIGESTIBLE NUTRIENTS

By C. C. BALCH, D. A. BALCH, S. BARTLETT, V. W. JOHNSON, S. J. ROWLAND and JILL TURNER

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(With 3 Figures)

Following reports in the literature, it has been confirmed that cows in the Institute herd will produce milk of low fat content if given diets containing 8 lb. or less hay daily and considerable amounts of more concentrated foods(1,2,3). There have been suggestions that low milk fat contents are caused by a shortage of roughage in the diet rather than by an excess of more readily digestible nutrients. It seems reasonable to suppose that in either instance the amount of nutrients digested is of greater importance than the amount of nutrients consumed.

There was no previous record of the intake of digestible nutrients in cows in which the milk fat content was depressed by diets of this type. Digestibility trials were therefore conducted on five cows which received during successive periods, (*a*) a normal diet of hay and concentrates, (*b*) a diet, consisting of 4 lb. hay with concentrates, which did not depress the milk fat content, and (*c*) a diet, of 4 lb. hay and concentrates of another type, which markedly depressed the milk fat content. The results have provided valuable information on the differences in the intake of digestible nutrients between normal diets and diets producing low milk fat contents.

The marked differences in the three diets might be expected to influence the mechanical processes of digestion, and accordingly the rate of passage of hay through the digestive tract was determined.

METHOD

The experiment lasted 18 weeks and was conducted on five Shorthorn cows which had calved 52–73 days before it began.

Foods. The cows were fed throughout on diets composed of hay and one of two types of concentrates. The hay consisted mainly of lucerne with a small proportion of cocksfoot and had been harvested in good condition by using tripods. One of the concentrates was of the wartime type, still widely manufactured at the time of the experiment, and sold in the form of cubes as National Cattle Food no. 1. The constituents of this mixture are given in Table 1; in quality it was probably below that of many of the dairy cubes now available. For convenience this mixture will be referred to as the 'concentrate cube', but this should not be taken to imply that the cubing process had any influence on the observed results. The second concentrate mixture (Table 1) was of the simple type used in earlier experiments of this series and will be called the 'concentrate mixture'.

Table 1. *The constituents of the concentrates* (%)

Concentrate cubes*		Concentrate mixture*	
Wheat feed	20	Flaked maize	50
Barley meal	25	Wheat feed	35
Maize meal	5	Decorticated groundnut meal	15
Molasses	7·5	Mineral mixture	1 lb./cwt.
Extracted decorticated sunflower meal	10		
Sugar-beet pulp	5		
Undecorticated cotton cake meal	12·5		
Copra cake meal	7·5		
Proprietary vegetable protein concentrate	7·5		

* See p. 305.

Treatments. The experiment was divided into four periods with the following diets:

Period I (preliminary control), weeks 1–4. The cows received daily 20 lb. hay, with slight adjustments to meet the needs of cows of varying weight and appetite, and the concentrate cube at the rate of approximately 4·5 lb./10 lb. milk produced.

Period II (first experimental period), weeks 5–7. Week 5 was devoted to a gradual change of diet, at the end of which the hay intake of the cows had been reduced to 4 lb. per day and the cubes increased by 6–10 lb. above the amount given during period I. Appetite was a limiting factor in adjusting the amounts of cubes given.

Period III (second experimental period), weeks 8–13. The daily hay allowance was kept at 4 lb., but the cubes were replaced by a rather smaller amount of the concentrate mixture.

Period IV (final control), weeks 14–18. The daily allowance of hay was raised to 18 lb., and the concentrate mixture was given at the rate of 4–5 lb./10 lb. milk produced.

Digestibility trials. In periods I–III the cows wore harness and bags for the separation of faeces and urine (4) during collection periods each lasting 10 days. From the data accumulated in these periods the digestibility of various nutrients was calculated according to procedures already described (5). The collection periods occupied part of weeks 3–4, 6–7 and 11–12.

Rate of passage of hay. On the first day of the collection periods the cows were fed small meals of hay stained both red and green and the numbers of undigested stained particles excreted in subsequent voidings of faeces were determined. Details of this method have also been published (5).

Analytical procedures

Foods and faeces. The conventional determinations on foods and faeces were made by standard methods. Cellulose was determined by the method of Norman & Jenkins (6), and pentosans by distillation with 12% HCl (7) saturated with sodium chloride, the resultant furfuraldehyde being determined by the method of Hughes & Acree (8). Values for pentosans were calculated as xylan using a pre-determined conversion factor for furfuraldehyde to xylose of 1·71. Both total pentosans and pentosans in cellulose were determined, and the resulting values are reported as cellulosans (the pentosans associated with cellulose), and as pentosans not in cellulose (the difference between the determined values for total pentosans and cellulosans). Starch was determined by the method of Pucher, Leavenworth & Vickery (9).

Milk. Three composite evening/morning samples of the milk of each cow were taken on alternate days in each week. The fat content of the samples was determined by the Gerber method (10), and the total solids content of one of the samples each week by a gravimetric method ((11), method 2), and the solids-not-fat obtained by difference.

Starch equivalent. The starch equivalent of the foods was calculated from the percentage of digestible nutrients found. Values for the digestibility of the hay fed alone were found in a subsequent trial with the same cows; the digestibility of the concentrates could therefore be calculated. In converting to units of starch equivalent the factors used were 0·94 for digestible crude protein, 2·12 and 1·91 for digestible ether extract in concentrates and hay respectively and 1·0 for digestible nitrogen-free extract and digestible fibre. The usual correction of 0·58 × percentage of crude fibre was subtracted from the values for hay and, in the absence of authentic '*V*' values a correction of 0·29 × percentage of crude fibre was subtracted from the values for the concentrates. Values for hay were further increased by 20% to allow comparison with the values given by Woodman in 'Rations for Livestock' (12).

RESULTS

Milk yield and composition. Mean values for the milk yield, milk fat content and weight of fat from the cows during the whole experiment are given in Fig. 1. It is clear that the changes in diet during period II caused no fall in the milk fat content, which remained at least as high as in the control period. Although this period only lasted 3 weeks, and may have been insufficient for recording a full response, the complete absence of any decline leaves little doubt that the conditions necessary to bring about the fall in milk fat were not present. However, when the concentrate cubes were replaced by the concentrate

Table 2. *Mean milk fat percentage in the* 2 *weeks which included each collection period and in weeks* 17 *and* 18

Cow	Period I, weeks 3 and 4	Period II, weeks 6 and 7	Period III, weeks 11 and 12	Period IV, weeks 17 and 18
1	4·10	4·37	3·87	4·46
2	4·03	3·84	2·22	3·86
3	4·75	4·87	3·76	4·72
4	3·58	3·85	2·88	4·09
5	3·74	3·64	2·28	3·61
Mean	4·04	4·11	3·00	4·15
		Change from period I		
1	—	+0·27	−0·23	+0·36
2	—	−0·19	−1·81	−0·17
3	—	+0·12	−0·99	−0·03
4	—	+0·27	−0·70	+0·51
5	—	−0·10	−1·46	−0·13
Mean	—	+0·07	−1·04	+0·11

mixture, there was an immediate fall in the fat content of the milk. The decline appeared to have commenced in composite samples of the milk produced 24 and 32 hr. after the change in diet. The mean fat percentage of the milk produced by individual cows in the fortnightly periods which included each digestibility trial are given in Table 2. On average, the fat content fell from 4·04% in period I to 3·00% in period III. This fall was least marked in cow 1, in which the fat percentage was only 0·23 lower in period III than in period I. The initial fat content was regained in the second week after changing back to normal amounts of hay.

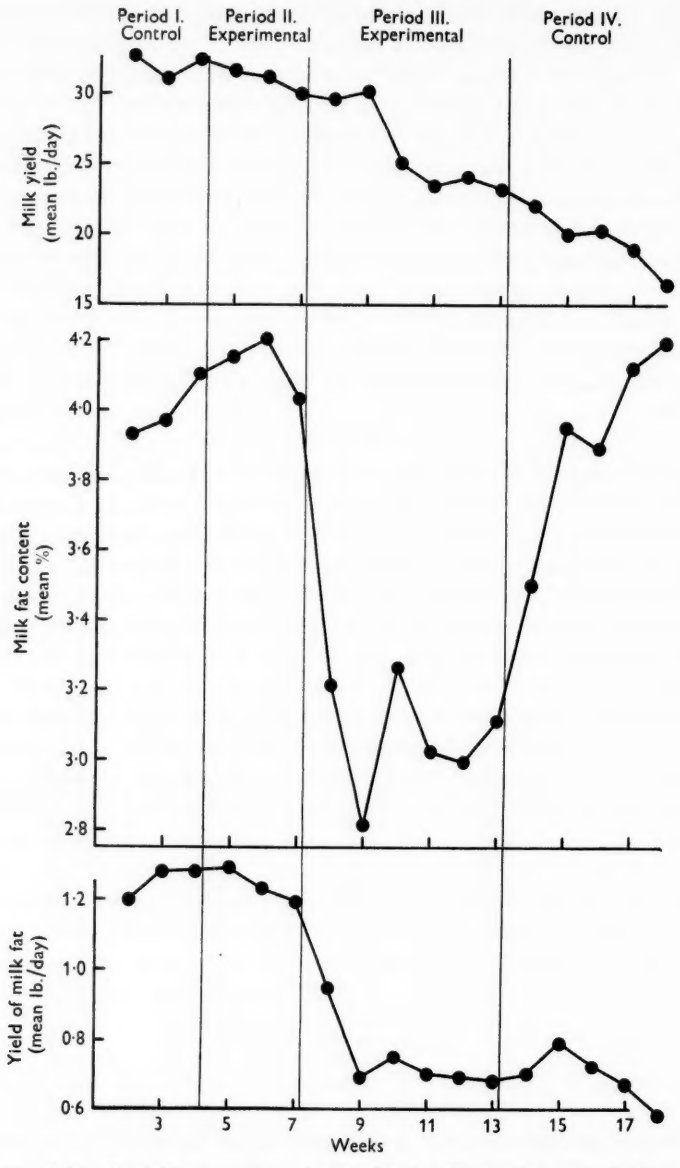

Fig. 1. The mean daily milk yield, fat content and yield of fat in milk from five cows. In the control periods the cows ate 17–21 lb. hay daily but in the experimental periods the hay was reduced to 4 lb. In the initial control period (period I) and in the first experimental period (II) the cows received concentrates in the form of dairy cubes and in the second experimental period (III) and in the final control period (IV) a concentrate mixture was given. Concentrates were rationed according to yield with additional amounts in the experimental periods.

Since the milk yield continued to decline at a fairly constant rate throughout the experiment, the fall in milk fat content during period III caused a marked and sudden decline in the weight of milk fat produced. Throughout period III (weeks 8–13) this represented a loss of approximately 30%.

The mean weekly values for the solids-not-fat content of the milk are given in Fig. 2. Here again there were not appreciable differences between periods I and II, but startling changes in period III. The mean percentages were solids-not-fat 8·74, 8·79, 9·22, and milk

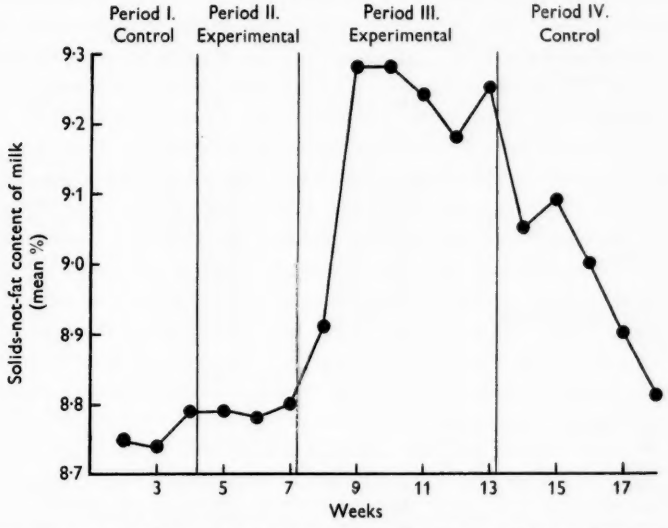

Fig. 2. The mean content of solids-not-fat in milk from five cows. Details of the treatments are given in the legend to Fig. 1.

protein 3·20, 3·24, 3·69, in the successive collection periods. During the final control period normal levels of solids-not-fat were regained less rapidly than normal levels of fat, and the recovery took about 5 weeks: by weeks 17 and 18 the mean percentage was 8·85. The increase in percentage in period III as compared with period I was observed in all the cows, varying from 0·21 to 0·69 with a mean of 0·48, and appears to have been due entirely to an increase in milk protein.

Table 3. *The composition of the foods* (%)

Food	Dry matter	Crude protein	Ether extract	Nitrogen-free extract	Crude fibre
Hay	83·0	11·38	1·32	32·54	30·01
Concentrate cubes	86·1	16·15	2·91	50·55	9·59
Concentrate mixture	83·5	15·88	2·58	57·36	4·61

	Cellulose	Cellulosans	Pentosans not in cellulose	Starch	Ash
Hay	30·83	7·34	7·49	0	7·78
Concentrate cubes	13·82	3·50	8·72	16·32	6·90
Concentrate mixture	9·38	4·00	6·55	30·48	3·07

Food intake and digestion. The composition of the foods is given in Table 3. The main differences between the composition of the two types of concentrates were that the concentrate mixture contained less than half as much crude fibre and almost twice as much

starch as the cubes, these differences being reflected respectively in the values for cellulose and nitrogen-free extract.

The amounts of food and of food constituents consumed and digested in the collection periods are summarized in Table 4. In the successive periods of the experiment the intake of dry matter, crude protein and ether extract declined, but in general the amounts

Table 4. *The mean amounts of food and food constituents (lb./10 days) consumed and digested by five cows in three periods of the experiment with the standard deviation of values for single cows*

Food and food constituents	Intake		
	Period I	Period II	Period III
Hay	189±16·5	40	40
Concentrates:			
Cubes	154±20·7	238±16·4	—
Mixture	—	—	222±20·5
Dry matter	289·2±21·1	238·1±14·1	218·2±17·6
Crude protein	47·1±3·7	42·2±2·6	40·0±3·4
Ether extract	7·55±0·67	6·98±0·44	6·18±0·54
Nitrogen-free extract	137·9±11·1	133·7±8·3	140·4±12·1
Crude fibre	71·9±5·1	35·9±1·6	21·4±1·0
Cellulose	81·1±5·6	46·3±2·3	31·8±2·0
Cellulosans	19·6±1·4	11·8±0·6	11·3±0·8
Pentosans not in cellulose	27·8±2·1	22·8±0·9	17·5±1·4
Starch	25·7±3·5	38·0±2·6	67·5±6·4
	Digested		
Hay	—	—	—
Concentrates:			
Cubes	—	—	—
Mixture	—	—	—
Dry matter	184·0±16·1	163·5±10·3	161·4±14·8
Crude protein	34·0±2·7	30·9±2·3	30·8±2·7
Ether extract	5·41±0·67	5·77±0·46	3·54±1·01
Nitrogen-free extract	98·4±9·0	101·5±5·3	115·1±10·2
Crude fibre	34·6±4·5	16·6±1·4	7·7±0·9
Cellulose	46·6±5·4	21·9±1·8	11·9±1·6
Cellulosans	10·8±1·5	7·3±0·6	7·6±0·4
Pentosans not in cellulose	17·8±1·1	13·6±0·7	9·0±1·1
Starch	25·7±3·5	38·0±2·6	67·5±6·4

digested fell to a small extent. The mean and standard deviation of the amounts of ether extract digested per cow in 10 days were 5·77 ± 0·46 lb. in period II, and 3·54 ± 1·01 lb. in period III. Although the intake of nitrogen-free extract remained relatively constant in the three periods, there was a tendency for the amounts digested to increase. The magnitude of the changes in the intake of these four fractions during period III does not seem large enough to offer an explanation for the changes in milk-fat production. However, marked differences did occur in the intake of crude fibre and of cellulose during the three dietary periods, and the amounts digested differed still more. During period I the mean amount of crude fibre digested per cow was 34·6 ± 4·5 lb./10 days, in period II it fell to 16·6 ± 1·4 lb., with no fall in milk fat content, and in period III it was only 7·7 ± 0·9 lb. The decreases for cellulose were of the same order while those for pentosans not in cellulose were rather less. Values for the amount of cellulosans digested were higher in period III than in period II and variations in this factor seem unlikely to be involved in the fall in milk fat. By far the largest increase in the amount of nutrient digested in period III as compared with period II was shown by starch, the mean amounts being 25·7 ± 3·5, 38·0 ± 2·6 and 67·5 ± 6·4 lb./10 days in successive periods.

Digestibility coefficients for the various food constituents are given in Table 5. Much of the variation between values for the different cows was evidently due to their varying ratios of concentrates to hay even during any one period. It does, however, appear that the change to 4 lb. hay daily depressed the digestibility of crude fibre, cellulose and pentosans not in cellulose, particularly with the concentrate mixture. No similar depression occurred in the digestibility of cellulosans, values for which were higher in periods II and III than in period I. During period II the digestibility of ether extract was higher than in period I but in period III it fell markedly. As is shown in Table 4, the changes in the intake of ether extract were only slight, the mean values being 7·5, 7·0 and 6·2 lb./cow/10 days in periods I–III respectively. The changes in digestibility and in the amount apparently digested were due to variations in the faecal excretion; in the three periods the mean amounts of ether extract excreted were 2·1, 1·2 and 2·6 lb./10 days.

Nutritive value of the diets. Values for the starch equivalent of the foods, calculated from their determined content of digestible nutrients, are given in Table 6. Values for the daily intake of starch equivalent and digestible crude protein are also given and compared with the commonly used standard values of requirements, based on the size of the cow and the amount and fat percentage of the milk, given by Woodman (12).

Alternative values for the starch equivalent of the two concentrates were calculated from the known proportions of their constituents and the typical values for the starch equivalent of these constituents as given in Woodman's tables, namely 60 for the concentrate cubes and 68 for the concentrate mixture. Our determined values for these foods were 56 and 62 respectively. These differences between values from the tables and those obtained by digestibility trial will have been due largely to the lower digestibility coefficients found for some nutrients, and especially for crude fibre, with the diets of hay and concentrates and those found with the all-hay diet. By the method of calculation of starch equivalent the whole of this decrease in starch equivalent must necessarily fall on the concentrates of the mixed diets, because the digestibility coefficients for the hay were presumed to be unchanged from those obtained with an all-hay diet. However, the similarity of the values for the cubes, obtained with the very different diets in periods I and II (56·3 and 56·5), encourages confidence in the accuracy of the values.

In view of the need to maintain the amounts of the diets constant for considerable periods of time, the rationing of the cows proved to be surprisingly close to the standard requirements for energy. In period III the fall in milk fat content so reduced the requirements that there was apparently a mean daily excess of 3·2 lb. starch equivalent per cow. However, if the fat content of the milk produced by the cows in that period had remained the same as in period II the excess would have been 2·0 lb., or less than the requirements for a gallon of milk. The nitrogen balances (Table 6) confirm that the cows were fed closely according to requirements and that little breakdown of body reserves occurred. During periods II and III there were losses in body weight varying from 30 to 110 lb.: these were probably due to an emptying of the digestive tract, and in view of the other evidence it is unlikely that they represent a real loss of body substance.

In planning this experiment it was realized that without introducing an extra concentrate low in protein, it would be difficult to lower the proportion of hay to concentrates without exceeding the requirements for digestible crude protein. The values given in Table 6 show, however, that the daily surplus in period I, 0·67 lb., was greater than in period II, 0·47 lb. The daily surplus in period III was 1·07 lb. but if the milk fat per-

Table 5. *The digestibility coefficients for the three diets*

Period of experiment	Cow	Diet. Daily intake (lb.) Hay	Concentrates Cubes	Concentrates Mixture	Digestibility coefficients (%) Dry matter	Crude protein	Ether extract	Nitrogen-free extract	Crude fibre	Cellulose	Cellulosans	Pentosans not in cellulose	Starch
I	1	20	15	—	64·8	73·3	72·7	72·2	50·7	59·6	54·7	63·3	100
	2	16·8	15	—	61·9	72·1	69·3	69·8	43·7	51·6	46·4	66·4	100
	3	21	14	—	63·6	71·9	70·0	70·9	50·8	59·0	53·7	67·8	100
	4	18	14	—	63·1	71·7	70·0	71·5	46·2	58·0	59·5	62·7	100
	5	18·8	19	—	64·4	71·9	75·3	72·4	48·7	58·1	60·3	60·4	100
				Mean	63·6	72·2	71·5	71·4	48·0	57·3	54·9	64·1	100
II	1	4	25	—	70·9	76·1	85·5	77·0	47·8	51·2	66·1	62·2	100
	2	4	25	—	67·5	73·4	84·2	73·9	44·7	47·2	61·9	54·5	100
	3	4	22	—	68·7	74·1	84·7	76·6	44·1	47·5	59·7	62·1	100
	4	4	22	—	68·6	71·5	79·0	77·6	44·4	45·2	59·7	61·7	100
	5	4	25	—	67·9	71·0	79·9	74·8	49·0	45·6	62·2	56·7	100
				Mean	68·7	73·2	82·7	76·0	46·0	47·3	61·9	59·4	100
III	1	4	—	24	75·3	76·1	60·8	82·7	41·1	39·3	67·4	57·5	100
	2	4	—	24	74·6	77·2	71·2	82·1	35·5	40·8	65·2	45·2	100
	3	4	—	20	73·3	74·5	65·9	82·3	32·3	36·1	67·9	54·4	100
	4	4	—	20	73·0	78·6	36·4	81·4	36·1	33·9	70·1	49·1	100
	5	4	—	23	73·6	78·4	49·8	81·1	34·1	35·7	67·4	50·2	100
				Mean	74·0	77·0	56·8	81·9	35·8	37·2	67·6	51·3	100

Table 6. *The nutritive value of the three diets*

Period of experiment	Cow	Diet. Daily intake (lb.) Hay	Concentrates Cubes	Concentrates Mixture	Starch equivalent: Units per 100 units food Hay	Units per 100 units food Concentrates	Daily intake (lb.) Hay	Daily intake (lb.) Concentrates	Daily intake (lb.) Total	Daily requirement (lb.) Maintenance	Daily requirement (lb.) Milk	Daily requirement (lb.) Total	Digestible crude protein: Daily intake (lb.)	Daily requirement (lb.) Maintenance	Daily requirement (lb.) Milk	Daily requirement (lb.) Total	Nitrogen balance (g./day)
I	1	20	15	—	33·9	58·1	6·8	8·7	15·5	7·3	8·8	16·1	3·50	0·78	2·13	2·91	+18·6
	2	16·8	15	—	32·8	52·7	5·5	7·9	13·4	6·6	8·9	15·5	3·17	0·72	2·16	2·88	+17·2
	3	21	14	—	31·3	59·9	6·6	8·4	15·0	7·6	8·0	15·6	3·40	0·81	2·00	2·81	+16·8
	4	18	14	—	33·2	55·1	6·0	7·7	13·7	6·4	6·4	12·8	3·14	0·69	1·54	2·23	+5·4
	5	18·8	19	—	35·0	55·5	6·6	10·5	17·1	7·3	8·5	15·8	3·80	0·78	2·04	2·82	+37·6
	Mean				33·2	56·3	—	—	14·9	—	—	15·2	3·40	—	—	2·73	+19·1
II	1	4	25	—	33·1	57·9	1·3	14·5	15·8	7·3	8·7	16·0	3·36	0·78	2·11	2·89	−0·9
	2	4	25	—	30·2	55·3	1·2	13·8	15·0	6·6	8·8	15·4	3·23	0·72	2·12	2·84	−0·5
	3	4	22	—	30·5	57·2	1·2	12·6	13·8	7·6	7·1	14·7	2·91	0·81	1·77	2·58	−1·8
	4	4	22	—	32·4	56·7	1·3	12·5	13·8	6·4	6·1	12·5	2·81	0·69	1·48	2·17	+13·6
	5	4	25	—	34·2	55·5	1·4	13·9	15·3	7·3	7·7	15·0	3·13	0·78	1·85	2·63	−7·7
	Mean				32·1	56·5	—	—	14·7	—	—	14·7	3·09	—	—	2·62	−0·5
III	1	4	—	24	35·4	63·3	1·4	15·2	16·6	7·3	6·0	13·0	3·27	0·78	1·44	2·22	+18·6
	2	4	—	24	34·3	63·3	1·4	15·2	16·6	6·6	5·2	11·8	3·32	0·72	1·40	2·12	+24·0
	3	4	—	20	32·8	62·3	1·3	12·5	13·8	7·6	6·1	13·7	2·71	0·81	1·46	2·27	+1·4
	4	4	—	20	34·6	60·8	1·4	12·2	13·6	6·4	4·0	10·4	2·86	0·69	0·99	1·68	−9·1
	5	4	—	23	36·4	61·2	1·5	14·1	15·6	7·3	3·6	10·9	3·25	0·78	0·95	1·73	+13·2
	Mean				34·7	62·2	—	—	15·2	—	—	12·0	3·08	—	—	2·01	+9·6

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centage had remained as in period II it would have been only 0·92 lb. This shows that the amount of digestible crude protein in excess of requirements was little greater in period III than in period I, and was probably unimportant as previous work has shown that the level of protein intake is not closely related to the fall in milk fat content(2).

The rate of passage of hay. Changes in the consistency of the gut contents, owing to decreases in the amount of hay and increases in the amount of concentrates given, would be expected to influence the mechanical processes of digestion. Any changes would probably be reflected in the rate of excretion of undigested residues.

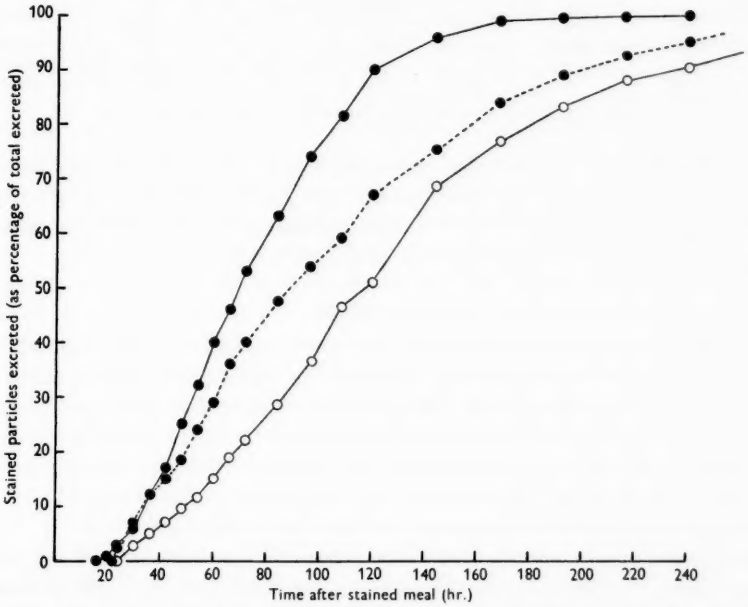

Fig. 3. Values for the excretion by cow 4 of undigested hay residues at intervals after feeding. The curves were obtained when the cow was receiving (*a*) 18 lb. hay and concentrate cubes, ●—● (period I); (*b*) 4 lb. hay and concentrate cubes, ●- - - -● (period II), and (*c*) 4 lb. hay and a concentrate mixture, ○—○ (period III). Excretion is shown as the percentage of the total stained particles excreted after a stained meal.

After a meal of stained hay the appearance of stained particles in the faeces follows a definite pattern. When the total number of particles excreted before the time of each sampling are plotted, the curve is of a sigmoid shape. For purposes of comparison, values are usually expressed as percentages of the total number of particles excreted in 10 days. It has been shown(5) that the initial appearance of stained particles in the faeces, or more reliably, the time required for excretion of 5% of the residues (called 5% excretion time), is indicative of the time taken by undigested residues to pass through the omasum, abomasum and intestines. Similarly, the spread of the excretion curve, which is shown by the difference between the times at which 80 and 5% of the residues are excreted (called 80–5% excretion time) is indicative of the time food has remained in the reticulo-rumen. Mean values for these indices of the rate of excretion are given in Table 7. The most noticeable feature was that the excretion curves became extremely prolonged with the diets low in hay, particularly in period III, and excretion was often prolonged after

the 10-day collection periods. Typical excretion curves, those for cow 4, are shown in Fig. 3. It was clear, however, that although this tendency slightly influenced the 5% excretion times, it was not apparent in the times for the first appearance of the stained residues in the faeces. In the periods of low-hay intake the faeces had a pasty consistency

Table 7. *Mean rate of excretion of hay by five cows, with the standard deviation of values for single cows*

	Diet. Average daily intake (lb.)			Time required for excretion of stained hay (hr.)			
		Concentrates					
Period of experiment	Hay	Cubes	Mixture	First appearance in faeces	5%*	80%*	80–5%*
I	18·9	15·4	—	18·4±3·3	27·6±3·9	94·2± 9·4	66·6± 8·1
II	4·0	23·8	—	16·4±4·6	28·8±4·7	149·4±24·9	120·6±24·4
III	4·0	—	22·2	19·2±3·3	33·0±8·5	163·4±37·5	130·4±29·2

* For explanation of these indices of the rate of excretion see p. 313.

which might have been taken to indicate a rapid rate of passage at least through the intestines. The excretion curves show that this was not true. The 80–5% excretion times were the longest yet found by us and show that roughage, in diets which contained little roughage, spent an abnormally long time in the reticulo-rumen.

DISCUSSION

This experiment has provided an observation of considerable practical interest, namely that even a marked reduction in the amount of hay, in a mixed diet of hay and concentrates, will not depress the milk fat percentage, even though there are simultaneous increases in the amount of concentrates given, unless the concentrates are of a certain type. Although Powell(13) appreciated that the constituents of the concentrate mixture were probably important, little attention has been given to this point and in very few experiments have details of concentrate mixtures been published.

In the present experiment two diets, each containing concentrates and 4 lb. hay have been compared. A diet in which the concentrates were given as a cube containing a variety of constituents did not influence the milk fat, but the change to a diet containing concentrates composed of flaked maize (50%), weatings (35%) and groundnut cake (15%) caused a mean fall in milk fat content from 4·04 to 3·00%. A comparison of the results of digestibility trials made while the cows were receiving these diets, and during an initial control period on a more normal diet in which they ate about 19 lb. hay daily, indicated the nutritional changes responsible for the fall in milk fat content.

The results of this experiment, coupled with the results of previous experiments in this series, lead to the conclusion that the essential feature of the diets that depressed milk fat content was not simply a low roughage or crude fibre content *per se*. The increased depression in milk fat content with a diet of 8 lb. ground hay, as compared with 8 lb. long hay, given with concentrates(3), the failure of additional crude fibre in the form of finely divided straw pulp to improve milk fat content(2), and further similar results as yet unpublished, show that a consistent, and presumably essential, characteristic of diets that depress fat content is the lack or absence of the physical property of 'fibrousness'—using the term in its ordinary and not in a strictly chemical sense. This, however, was clearly not the only essential feature, for in the present experiment there was no fall in milk fat content with a diet of 4 lb. of hay plus concentrate cubes, but there was an

immediate fall in fat on changing from the cubes to the concentrate mixture, although the 'fibrousness' of the two diets must have been closely similar. The cubes contained more crude fibre, finely divided, but the marked result of the change in concentrates was in the intake of digestible starch. It is clear, therefore, that diets which depress milk fat content possess two essential characteristics: they provide a large intake of starch, or possibly other highly digestible carbohydrates, at a time when the diet lacks the physical property of fibrousness.

Following the first experiments of Powell (14) it was suggested that the depressions of milk fat content resulting from changes in diet may be associated with a disturbance of the functioning of the rumen, and this seems highly probable. Dietary roughage is known to be important for the maintenance of ruminal motility and rumination while starch, particularly the starch of maize, is known to have marked effects on the digestive processes of ruminants. In this experiment the starch in the concentrate mixture was derived largely from flaked maize, whereas the concentrate cubes contained only 5% maize products. The depression of fibre digestibility observed with the diet high in maize starch almost certainly indicates a disturbance of digestion in the reticulo-rumen and is in agreement with a number of experiments conducted elsewhere, and recently reviewed by Head (15) on non-lactating sheep and cattle. Flaked maize is known to have marked effects on the growth of rumen bacteria (16) and may have played some special role in the present series of experiments. It is, therefore, highly probable that the depression of milk fat content by certain diets arises from changes in the physical and biochemical processes taking place in the reticulo-rumen.

The intake of ether extract varied only slightly in the three diets, but there were appreciable differences in the amounts digested. For the low hay diet, with concentrate cubes, the apparent digestibility coefficients were higher than for the control diet. This would be expected to follow a fall in the amount of roughage in the diet; the smaller amount of ether extract appearing in the faeces under such conditions has been ascribed partly to a reduced removal of the intestinal lining (17). It was, therefore, surprising to find that with the diet of low hay and the concentrate mixture the amount of ether extract excreted was higher than with either of the other diets, in spite of the smaller intake. No explanation for this change is yet available. It is unlikely that the fall in the intake of digestible ether extract can have played a part in bringing about the depression in milk fat content because at the time of this depression the intake of digestible ether extract per 10 lb. of milk produced (0·15 lb.) was scarcely less than in the control period (0·17 lb.) or in the period of low hay with concentrate cubes (0·19 lb.).

When the nutritive value of the foods was calculated from their content of digestible nutrients it appeared that the cows were fed in close accordance with their requirements for energy (starch equivalent) as indicated by the usual standards adopted in Great Britain, but they received rather more digestible protein in all periods than is provided by these standards. However, if values for the daily intake of starch equivalent had been taken directly from Woodman's tables (12) the estimated intake would have been appreciably higher than the estimated requirements. That the energy intake was not excessive is an important point, for while it has been shown that very large falls in milk fat content can be produced by reducing the intake of hay to 6 lb. or less daily and giving concentrates *ad lib.* (18), it is now clear that moderate falls can be produced without over-feeding, a possibility that Loosli, Lucas & Maynard (19) had discussed earlier.

Changes in the rate of passage of undigested particles of stained hay, showed that when there was little hay in the diet, hay remained for an abnormally long time in the reticulo-rumen. In spite of this there was a marked fall in the digestibility of crude fibre when the concentrate mixture was given, providing further evidence that at the time of the depression in milk fat content, there was a change in the activity of the flora of the reticulo-rumen.

Powell(13,14) claimed that the solids-not-fat content of milk was lowered by diets low in hay, but other workers have failed to find any change. An undoubted increase of 0·48% was found in this experiment at the time of the fall in milk fat content, and was due to an increased milk protein content.

SUMMARY

1. Digestibility trials were conducted, and the rate of passage of hay was measured, with five Shorthorn cows during a period of normal diet and during two experimental periods in which diets low in hay and high in concentrates were given. In the first experimental period the concentrates were cubes of the wartime type sold as National Cattle Food No. 1, and containing a variety of constituents; in the second they were a mixture of flaked maize (50%), weatings (35%) and decorticated ground-nut cake (15%). For convenience these are referred to as concentrate 'cubes' and 'mixture' respectively.

2. During the initial and final control periods the cows consumed daily 17–21 lb. hay, and about 4·5 lb. concentrates per 10 lb. of milk produced. In the initial control period the concentrates were the concentrate cubes, and in the final they were the concentrate mixture. The hay was reduced to 4 lb. daily during the two experimental periods and the remainder of the standard requirements of the animals were met by concentrates. The concentrates were the cubes in the first experimental period and the mixture in the second experimental period.

3. Reducing the hay to 4 lb. did not affect the fat content of the milk when the other food in the diet was the concentrate cubes, but there was a striking mean fall of 1·04% fat when the cubes were replaced by the concentrate mixture. This represented a loss of over 30% in the yield of fat.

4. Digestibility trials, conducted in the initial control and first and second experimental periods, indicated that the fall in milk fat content was not the result of changes in the amounts of dry matter, crude protein, ether extract, crude fibre, cellulose, cellulosans or pentosans not in cellulose digested. The essential difference between the diet of low hay with the concentrate mixture and the other diets given in this experiment was that it provided a high intake of starch yet had little of the physical property of roughage. It is concluded that depression of milk fat content results from a combination of these two factors and probably originates from changes in the physical and biochemical processes of the reticulo-rumen.

5. The intake of starch equivalent, as calculated from the intake of digestible nutrients, was close to standard requirements in all periods of the experiments, but there was a surplus of digestible crude protein.

6. The mean solids-not-fat content of the milk rose 0·48% at the time of the fall in milk fat, and this was entirely due to an increase in milk protein. After the return to normal diets the recovery of solids-not-fat was slower, but no less complete, than the recovery in milk fat.

We wish to acknowledge the help and advice of the late Dr T. H. French in the planning of the experiment, and to thank Dr H. E. Woodman of Cambridge for advice on the calculation and interpretation of the values for starch equivalents. We also wish to thank Mr A. S. Foot and his colleagues of the Dairy Husbandry Department for their interest and help, Mr A. W. Wagstaff, Mr D. Millard and Miss R. E. Thorner for analytical work, Mr D. G. Kemp for much reliable work in the cowshed, and Mr F. Leaver for arranging the milking and milk sampling.

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558. THE SECRETION OF A SINGLE TRACER DOSE OF LABELLED IODIDE IN THE MILK OF THE LACTATING COW

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(With 1 Figure)

In a recent study on the influence of climatic conditions on the deposition of airborne radio-iodine vapour Chamberlain & Chadwick(1) note that the rate of removal of radio-iodine from herbage by translocation and leaching is small compared with the half life of the isotope. Thus in the event of an accidental emission of abnormally large quantities of fission-product iodine from nuclear energy plants cows grazing in the neighbourhood may ingest sufficient of the isotope to constitute a danger to the consumers of their milk.

There is an extensive literature of the fate of dietary and injected iodine in the animal body, but it is chiefly concerned with the metabolism of the thyroid gland (see, for example, review by Sachs(2)). In unpublished experiments from this laboratory it has been found that most of a single dose of labelled iodide injected into a sheep is excreted in the urine in the course of a week or so, although a small but appreciable fraction (about 2%) is excreted with the faeces. There is but scanty information available, however, about the secretion of dietary iodine, especially at tracer dose levels, into the milk of ruminants, and it has therefore seemed desirable to investigate it.

1. RATE OF APPEARANCE IN MILK

Experimental

Animal. The cow, was a Shorthorn aged 12 years and in her 9th lactation. Though old her general condition was good and her milk yield average for the herd, namely about 700 gal. per lactation. She was in her 7th month of lactation when this experiment was started. During the experiment she was kept in a box and fed on concentrates mixture and hay with water *ad lib.*

Milk collection. The cow was hand milked at the usual times morning and afternoon, the milk measured and the whole sample sent to the laboratory for assay. The observed activity as measured by the liquid counter was converted to specific activity by means of the factor quoted below, and the total activity in the whole sample calculated.

Counter. The counter used was a 20th Century type M6 liquid counter. It was found by counting the reference sample that 100 counts/min. corresponded with a specific activity of 0·485 μc./l. of solution.

Results. The results obtained over a period of 15 days are shown in Table 1. The maximum specific activity of the milk occurred on the first day after dosing, and by the 15th day it had fallen to about 0·5% of its maximum value. This was an activity about equal to the background of the counter below which accurate measurements are difficult

to make. This period embraces, however, a decline of more than two orders of magnitude in the specific activity of the milk.

It will be seen that columns II–IV refer to the *observed* radioactivity in the milk uncorrected for the decay of the isotope, the figures in column IV showing the total observed radioactivity in each day's milk. Columns V–VII refer to the radioactivity in the milk

Table 1. *Rate of appearance of a single* 10 *mc. dose of* ^{131}I *administered carrier free as* KI *to a lactating cow*

(Figures in columns II–IV uncorrected, figures in columns V–VII corrected for decay of isotope. (Half life = 8 days). 349 and 476·9 μc. correspond with 3·49 and 4·769% respectively of original dose.)

		Activity (uncorrected)			Activity (corrected)		
Day	Volume of milk (l.) I	Specific activity (μc./l.) II	Total activity in sample (μc.) III	Total activity for day (μc.) IV	Specific activity (μc./l.) V	Total activity in sample (μc.) VI	Total activity for day (μc.) VII
0	—	—	—	—	—	—	—
1	5·00	15·0	75·0	119·0	17·5	87·0	137·0
	2·50	17·8	44·5	—	20·5	50·0	—
2	5·00	12·4	62·0	89·5	15·7	79·0	114·0
	2·28	12·1	27·5	—	15·3	35·0	—
3	4·10	7·76	31·8	56·3	10·7	44·0	77·5
	3·18	7·71	24·5	—	10·6	33·5	—
4	4·66	4·80	22·3	35·8	7·20	33·4	53·7
	3·18	4·26	13·5	—	6·40	20·3	—
5	5·00	2·46	12·3	17·9	3·98	20·0	29·2
	3·18	1·78	5·60	—	2·90	9·20	—
6	5·22	1·31	7·00	11·1	2·34	12·3	19·7
	3·07	1·35	4·10	—	2·41	7·40	—
7	4·76	0·810	3·80	6·20	1·59	7·50	12·1
	2·95	0·800	2·40	—	1·55	4·60	—
8	4·76	0·530	2·50	3·80	1·13	5·30	8·00
	3·18	0·400	1·30	—	0·860	2·70	—
9	5·00	0·280	1·40	2·15	0·650	3·20	5·00
	2·50	0·300	0·75	—	0·710	1·80	—
10	5·00	0·256	1·31	1·87	0·660	3·30	4·70
	3·07	0·184	0·560	—	0·480	1·40	—
11	4·76	0·190	0·900	1·31	0·530	2·50	3·70
	2·95	0·150	0·410	—	0·410	1·20	—
12	4·55	0·135	0·610	0·980	0·420	1·90	3·04
	2·73	0·135	0·370	—	0·420	1·14	—
13	4·55	0·121	0·550	0·930	0·406	1·85	3·15
	2·73	0·141	0·380	—	0·475	1·30	—
14	4·76	0·126	0·600	0·910	0·460	2·20	3·35
	2·50	0·126	0·310	—	0·460	1·15	—
15	4·55	0·102	0·475	0·720	0·400	1·82	2·80
	2·50	0·097	0·242	—	0·390	0·980	—
Total activity secreted (μc.)			—	349·0	—	—	476·9

corrected to what would have been observed if the isotope did not decay. Although expressed in the same units therefore as the figures in column III those in column VII are a measure of the rate of secretion of labelled iodide in the milk independent of the nature and rate of decay of the isotope used as a tracer.

It will be seen that the concentration of labelled iodide in the milk reached a peak on the second milking of the first day when it had a value of 20·5 μc./l. This is to be compared with a value of 25 μc./l. which would be expected if the original dose of 10 mc. had been

diluted with an amount of water equal to the estimated water content of a 540 kg. animal (say 400 kg.).

Over the 15 days of the experiment the total amount of labelled iodide secreted in the milk was, corrected for decay, 4·77 % of the administered dose although decay reduced the observed value to 3·49 %. The progress of this secretion is shown in Fig. 1 in which the total secretion of labelled iodide and of radioactivity is plotted semilogarithmically. The upper curve (●—●—●) shows the secretion of labelled iodide and it will be seen that between the 1st and 9th day the plot is almost exactly linear. During this period the

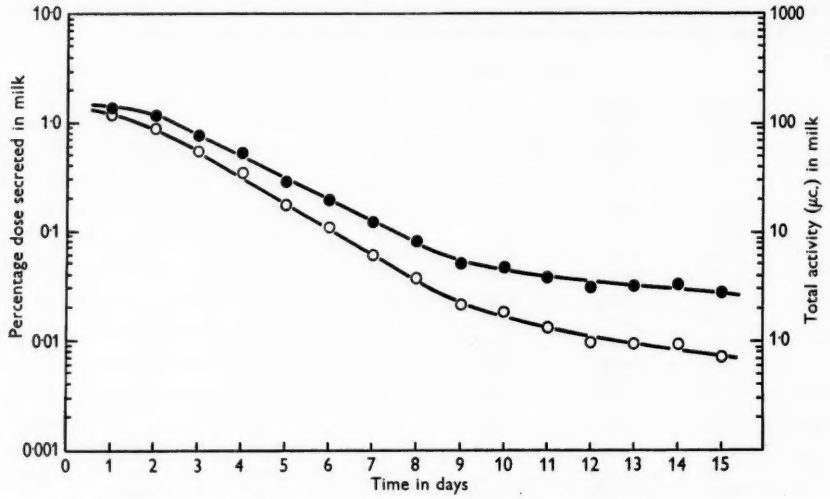

Fig. 1. Secretion of radioactivity in milk of dairy cow after single oral dose of 10 mc. ^{131}I. ●—●, activity corrected for decay; ○—○, observed activity.

amount of labelled iodide secreted in the milk per day declined by a factor of about 25: i.e. from 1·3 to 0·05 % of the original dose. The slope of the curve over this period shows that secretion of labelled iodide in milk diminished with a half period of about 1·5 days. At the 9th day the curve begins to flatten out and thereafter has a smaller slope. This obviously corresponds with a much slower process than that governing the secretion of the bulk of the dose and is probably the release, by exchange or degradation, of iodine from chemical combination. One possible explanation would be that this portion of the curve corresponds with the release of iodine from combination in the thyroid gland as its place is taken by new and unlabelled iodine from the diet.

The lower curve on Fig. 1 (○—○—○) shows the secretion of radioactivity uncorrected for decay. It is the relevant curve where radiation hazards are the main consideration. It represents, of course, the sum of two exponentials: the exponential secretion of labelled iodine as indicated in the upper curve plus the exponential decay of the element itself. This being so it is not a straight line but approaches close enough to linearity for the half period to be reckoned as approximately 1·2 days.

From these results it is possible to predict what would be observed if, instead of a single dose, the animal received a regular daily dose of the same size. From the linear part of the lower graph it will be seen that the total activity in the milk declines each day to about

0·57 times the previous day's level. The daily administration of radio-iodine would therefore cause the radioactivity in the milk to increase until it reached a level where the daily loss was exactly balanced by the daily intake. This works out at 2·3 times the maximum level arising from a single dose and would be reached in about 1 week. If the daily intake then ceased the total activity in each day's milk would diminish with the half period of about 1·2 days shown in this experiment.

No provision was made for the regular collection of urine but samples were assayed on two occasions chiefly to ascertain whether it was safe to graze the animal on pastures to which the rest of the herd had access. On these two occasions the specific activity of the urine was considerably higher than that of the milk. Thus on day 8, when the specific activity of the milk had declined from its peak value of 17·8 μc./l. to 0·48 μc./l. the specific activity of the urine was 4·4 μc./l.—that is nearly 10 times higher than that of the milk. By the 14th day the specific activity of the milk had declined to 0·13 μc./l. but the specific activity of the urine was 0·25 μc./l.,—i.e. nearly double.

These results would thus appear to be in agreement with the conception that iodide follows a similar path to chloride, much more being lost in the urine than in the milk.

2. CHEMICAL NATURE OF THE RADIOACTIVE SUBSTANCE IN THE MILK

To 10 ml. milk (sample 2) was added 10 mg. inactive potassium iodide as carrier and the proteins and fat precipitated by the usual analytical procedure. The filtrate was made up to 50 ml. On assaying in the counter and correcting the result for the dilution it was found that all the activity was in the filtrate showing that none was in combination with fat or protein.

10 ml. of the solution were then acidified and an excess of silver nitrate added. The precipitate was filtered off, the filtrate concentrated and made up to 10 ml. It was found to contain no radioactivity. It thus appears that the radioactivity in the milk follows the chemistry of the iodide ion which is in the form in which it was administered to the animal.

SUMMARY

1. A tracer dose of ^{131}I in the form of radioactive potassium iodide of about 50 μg. in weight has been administered to a lactating cow and the secretion of the radioactivity in the milk studied.

2. The highest activity was found in the milk on the 1st day after dosing and was only slightly lower than that expected if the iodide had been uniformly distributed throughout all the water in the animal's body.

3. The secretion of labelled iodine in the milk attained a maximum on the 1st day after dosing and thereafter declined exponentially with a half period of 1·5 days until the 9th day. The rate of secretion, though still approximately exponential, then had a much longer half period (about 10 days) but was too small to study at the dose level used.

4. Owing to the decay of the element the observed secretion of radioactivity declined more rapidly than that calculated for labelled iodine and over the first 9 days of the experiment had a half period of approximately 1·2 days. Thereafter the radioactivity declined more slowly with a half period of approximately 3·5 days.

5. From these findings it is calculated that regular daily dosing at the same rate would result in an equilibrium level in the milk about 2·3 times the maximum arising from a single dose.

6. Less than 5% of the original dose was recovered from the milk over the period of the experiment by which time the concentration had declined to 0·5% of its maximum.

7. The radioactivity in the milk was found to be in the chemical form of iodide ion.

Thanks are due to Mr B. B. Brown for supervision of the care and milking of the animal.

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559. THE INHIBITION OF MICRO-ORGANISMS BY RAW MILK

III. DISTRIBUTION AND PROPERTIES OF TWO INHIBITORY SUBSTANCES, LACTENIN 1 AND LACTENIN 2

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(With 8 Figures)

INTRODUCTION

The antibacterial properties of raw milk are known to be due mainly to bacteriostatic substances which can be destroyed by heating. Two substances, lactenin 1 (L_1) and lactenin 2 (L_2) have been found responsible for the inhibition of *Streptococcus pyogenes* by raw milk and methods have been described for their estimation (1). The existence of L_1 and L_2 was later confirmed by their separation by acetone at low temperature and the electrophoretic examination of the fractions was described (2). In the present paper various aspects of the inhibition by lactenin are considered, especially the epidemiology of mastitis and the role of lactenin in the keeping quality of pasteurized milk.

Jones & Little (3) were first to suggest that lactenin might have a role in the resistance of cows to mastitis. Wilson & Rosenblum (4) have since shown that anaerobic conditions and reducing agents abolish the activity of lactenin on *Str. pyogenes*, and they expressed the opinion that because of the anaerobic conditions of the udder, it was probable that the action of lactenin *in vivo* was very limited. However, other bacteria have different sensitivities to the two lactenins, and the action of L_1 or L_2 on different organisms is not necessarily abolished by anaerobic conditions.

Several authors (3,5) have already searched for a relation between the lactenin content of milk and the infection of the udder. Since either of the two substances L_1 and L_2 may be responsible for the inhibition of micro-organisms by milk, it seemed necessary to determine separately the content of L_1 and L_2 in milk. The distribution of L_1 and L_2 has therefore been studied in various samples of milk, taken in particular from separate quarters of cows known to be sensitive or resistant to mastitis.

Lactenin is not destroyed by pasteurization for 30 min. at 61° C. (6). It may therefore be assumed that lactenin contributes to the keeping quality of milk which has been pasteurized under these conditions. If the heat treatment has been different, especially if the milk has been overheated, lactenin may have been destroyed and the keeping quality of the milk reduced. The effect of pH and heat on the stabilities of L_1 and L_2 has already been described (1). In the present work the effects of heat on L_1 and L_2 have been studied for various combinations of time and temperature.

Although raw milk has been shown to be inhibitory for a number of bacteria (see the review of literature given by Jones & Little (3)) the inhibitory action of lactenin itself has

* This work was carried out at the National Institute for Research in Dairying, Shinfield, during the tenure of a British Council Scholarship.

been especially studied on *Str. pyogenes* (3,6). Other substances in milk may also be responsible for the inhibitory phenomena described in the literature. It is therefore important to know whether other organisms are sensitive to L_1 and L_2 and whether both substances must be present to give the inhibition, as is the case for *Str. pyogenes* (1). A few organisms, three mastitis organisms and one lactic acid streptococcus, have been tested in these experiments for their sensitivities to L_1 and L_2.

In order to investigate the mode of action of lactenin, the growth of *Str. pyogenes* has been studied in more detail. It has been found that hydrogen peroxide abolishes the activity of lactenin on this organism. The comparison between the inactivation of lactenin by cysteine and by hydrogen peroxide will be discussed.

EXPERIMENTAL

The distribution of L_1 and L_2 in various samples of milk

Method

Samples were taken at intervals over a period of 10 months from the bulk milk of a herd of forty cows. Samples were also taken from the separate quarters of several cows of two herds. For this latter investigation it was found convenient to use samples of foremilk taken for mastitis investigations*. They were taken aseptically from separate quarters at the evening milking.

Table 1. *The occurrence of L_1 and L_2 in bulk milk*

(Units of L_1 and L_2 per ml.)

	Date of sampling							
	20. ii.	2. iv.	7. viii.	12. ix.	24. ix.	2. x.	28. x.	10. xi.
L_1	110	100	100	150	150	100	80	80
L_2	70	60	150	50	120	110	100	140

For most of the cows two or more samples were examined at various intervals. Information was recorded concerning the date of calving, the number of calves and previous or present infections of the udder. From the number and the seriousness of the mastitis infections developed during their previous lactations, the cows were classified as very sensitive, sensitive and resistant. All samples were heated at 60° C. for 20 min. This was always sufficient, even with the bulk milk used, to ensure the relative sterility necessary for assay.

The method of assay has already been described (1). The same standard was used as in the previous experiments, and the results are recorded as the number of units of L_1 and L_2 per ml. of sample. They are the average of duplicate assays done on two different days. These duplicates were generally in good agreement. Where differences occurred the assays were repeated, and only concordant results were retained.

Results

Bulk milk. Table 1 shows some of the results obtained with bulk milk taken from February to November. The L_1 content varied from 80 to 150 units and the L_2 content from 50 to 150 units/ml. These variations did not seem to be related to the season of the year.

Milk from separate quarters. Table 2 shows a comparison between the L_1 and L_2 contents

* These samples were provided by Mr F. K. Neave, to whom we are greatly indebted.

of the foremilk from the separate quarters of two cows and the concentration found in the total milk from the corresponding quarters. No significant difference was found between foremilk and total milk from separate quarters. The results obtained with the foremilk therefore may be taken as indicating approximately the lactenin content of the total milk of the separate quarters.

Table 2. *Comparison between foremilk and total milk from the separate quarters of two cows*

(Units of L_1 and L_2 per ml.)

Cow	Quarter	Present infection	L_1		L_2	
			Fore-milk	Total milk	Fore-milk	Total milk
Opera, 3 calves, resistant, 3 months' lactation	RF	No infection	100	100	70	70
	LF	No infection	80	70	70	70
	RH	No infection	100	80	70	70
	LH	No infection	80	80	70	70
Nasturtium, 7 calves, sensitive, 7 months' lactation	RF	Streptococci	70	70	100	80
	LF	No infection	50	70	80	80
	RH	No infection	50	50	80	80
	LH	No infection	50	70	80	120

Mid-lactation milk. Table 3 shows the results obtained during lactation for several cows with different susceptibilities to mastitis. The L_1 content of sixty samples of mid-lactation milk (from cows in lactation for $2\frac{1}{2}$–10 months) varied between 20 and 220 units, fifty-five of the sixty samples varying between 70 and 150 units. The L_2 content showed greater variations: it varied from 40 to 340 units, fifty-four of sixty samples varying between 40 and 180 units.

It is evident that the infection in a quarter at the time of testing had no influence on the L_1 or on the L_2 contents of the milk of the quarter. Although there was no definite correlation between the L_1 or L_2 contents of the milk of a cow and its susceptibility as judged by its record of previous infections, it must be remarked that the highest L_2 content (340 units) was obtained with Jean (very sensitive), whereas the three cows classified as 'resistant' had a low L_2 content. The stage of lactation and the age of the cows (4–11 years) did not seem to be related to the L_1 or L_2 contents of the milk.

Milk obtained during the 'drying-off' period. Table 4 shows the results obtained for two cows at the time of drying-off. In the two cows tested the L_1 content of the milk was relatively high (80–280 units). The L_2 content was low for one cow (resistant to mastitis) and high for the other (very sensitive to mastitis).

Colostrum. In Table 5 results are given for two cows at the time of calving and for samples of colostrum taken from four different cows on the day of calving. They show that important changes in the concentration of L_1 and L_2 occurred at the time of calving. The colostrum had a very high content of L_1 (up to 2200 units) and a very low content of L_2. From the results obtained with the two cows tested, it can be seen that the milk became normal 2 or 3 days after calving.

Time-temperature destruction of L_1 *and* L_2

The pH and heat stabilities of L_1 and L_2 have already been studied (1). L_1 is most stable at pH 6·0–6·5, 90% being destroyed at 68° C., L_2 is most stable at pH 7·0, 95% being destroyed at 74° C. The heat-destruction of L_1 and L_2 in milk at its normal pH value (6·8) has been studied for various combinations of time and temperature.

Table 3. *The occurrence of L_1 and L_2 in the milk of cows in mid-lactation*

(Units of L_1 and L_2 per ml.)

Cow	Quarter	Present infections	1st sampling		2nd sampling	
			L_1	L_2	L_1	L_2
			($2\frac{1}{2}$ months lactation)		(3 months lactation)	
Dorris 9, 7 calves, sensitive	RF	No infection	150	150	150	120
	LF	No infection	100	120	150	100
	RH	Staphylococci	100	120	120	120
	LH	Group II streptococci	80	120	120	120
			($2\frac{1}{2}$ months lactation)		(6 months lactation)	
Clara 10, 9 calves, sensitive	RF	No infection	80	150	220	60
	LF	Staphylococci	100	180	150	60
	RH	No infection	100	180	150	50
	LH	Staphylococci	100	220	120	80
			(8 months lactation)		(10 months lactation)	
Jean, 5 calves, very sensitive	RF	No infection	110	340	70	70
	LF	No infection	150	340	70	90
	RH	Group III streptococci + staphylococci	190	280	70	110
	LH	Group III streptococci + staphylococci	150	250	70	100
			(3 months lactation)		(5 months lactation)	
Clarice, 5 calves, sensitive	RF	No infection	70	70	70	50
	LF	No infection	90	60	70	60
	RH	Staphylococci	90	60	60	50
	LH	No infection	80	40	70	50
			(8 months lactation)		(10 months lactation)	
Gertrude, 4 calves, sensitive	RF	Staphylococci	120	70	80	50
	LF	Group III streptococci	110	70	80	100
	RH	No infection	100	70	80	60
	LH	No infection	100	40	100	70
			(7 months lactation)		(9 months lactation)	
Janet, 5 calves, sensitive	RF	Group III streptococci	140	120	120	90
	LF	Group III streptococci	100	170	80	120
	RH	Group III streptococci	120	140	100	80
	LH	Group III streptococci	100	160	80	110
			(10 months lactation)			
Nellie, 5 calves, resistant	RF	No infection	110	60	Not done	
	LF	No infection	140	60		
	RH	No infection	150	60		
	LH	No infection	140	60		
			(10 months lactation)			
Betty, 5 calves, resistant	RF	No infection	80	100	Not done	
	LF	No infection	80	90		
	RH	No infection	90	100		
	LH	No infection	90	100		
			(5 months lactation)			
Dainty, 6 calves, resistant	RF	No infection	70	50	Not done	
	LF	No infection	70	40		
	RH	No infection	30	40		
	LH	No infection	20	40		

Technique

Separated bulk milk was distributed in 3 ml. quantities in aluminium tubes (12 × 1 cm.) stoppered with rubber bungs. These tubes were preheated to 60° C. for a few minutes and completely immersed in a water-bath at the required temperature (± 0·1° C.) for a given time. The tubes were then rapidly cooled by immersion in a water-bath at 0–2° C. Under these conditions the destruction of lactenin during the heating and cooling processes was negligible when the heat-treatment itself lasted more than 2 min. The milk was then removed from the tubes and assayed for the L_1 and L_2 content.

Table 4. *The occurrence of L_1 and L_2 during the drying-off period*

(Units of L_1 and L_2 per ml.)

Cow	Quarter	Present infection	Date of sampling: 10. x. L_1	10. x. L_2	13. x. ↓ L_1	13. x. ↓ L_2	16. x. L_1	16. x. L_2	19. x. (dry secretion) L_1	19. x. (dry secretion) L_2
Anabella,	RF	No infection	190	60	180	50	160	80	120	50
3 calves,	LF	No infection	220	70	110	50	120	60	120	20
resistant,	RH	No infection	170	60	90	40	150	70	180	40
dry on 15. x.	LH	No infection	140	80	120	50	250	60	—	20

Cow	Quarter	Present infection	Date of sampling: 10. x. L_1	10. x. L_2	13. x. ↓ L_1	13. x. ↓ L_2	16. x. L_1	16. x. L_2
Winnie 16,	RF	Group III streptococci	100	210	180	170	280	90
4 calves,	LF	No infection	100	210	280	140	100	120
very sensitive,	RH	Group III streptococci	150	210	220	210	80	210
dry on 15. x.	LH	Staphylococci	150	220	120	170	140	230

↓ = Time of drying-off.

Table 5. *The occurrence of L_1 and L_2 in the secretion of two cows at the time of calving and in several samples of colostrum*

(Units of L_1 and L_2 per ml.)

		Date of sampling: 11. ii.	13. ii. ↓	15. ii.	16. ii.	18. ii.	20. ii.
Pippin (4 quarters), calved 15. ii.	L_1	550	550	550	550	120	120
	L_2	20	20	6	6	50	60

		Date of sampling: 11. ii.	↓	12. ii.	13. ii.	15. ii.
Ivy II (4 quarters), calved 12. ii.	L_1	1500		1500	120	150
	L_2	20		6	40	120

		L_1	L_2
Colostrum (day of calving)	Sarah 2	2200	10
	Flora 86	1000	30
	Valiant	450	20
	Maid	1600	10

↓ = Calving time.

Results

Figs. 1 and 2 show the time-temperature curves for 50%, and more than 99% destruction of L_1 and L_2. A straight line joining the points of legal standards of pasteurization (63° C. for 30 min. and 71° C. for 15 sec.) has been drawn for comparison.

It appears that L_1 and L_2 were completely destroyed only by a heat-treatment which exceeded that used in normal pasteurization. Only 50% or less of L_1 was destroyed by pasteurization. L_2, which will be shown to be the most effective of the two substances against microorganisms, was destroyed only by heating to 74° C. for 20 min. or an equivalent treatment.

Action of L_1 and L_2 on different organisms

Method

The separate action of L_1 and L_2 on different organisms has been observed by measuring the acid production of these organisms in milk containing varying concentrations of L_1 and L_2. Only organisms able to acidify milk rapidly have been used in these experiments.

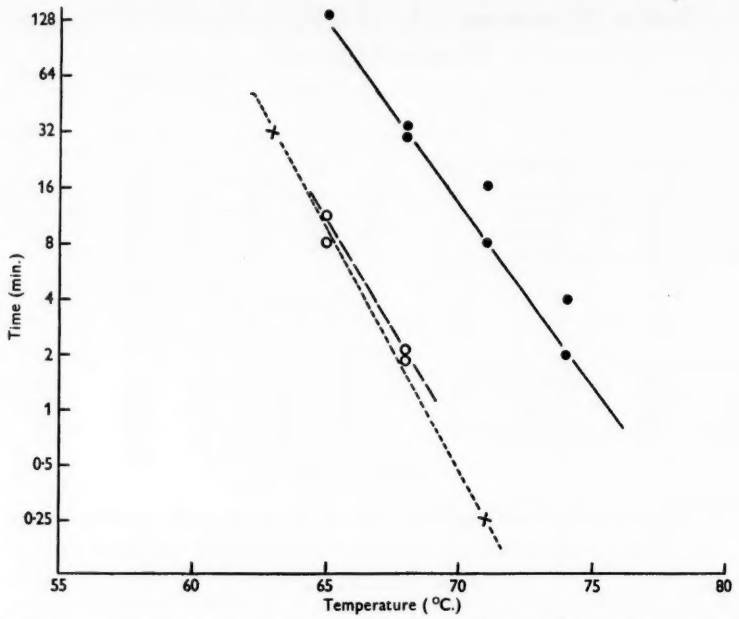

Fig. 1. The time-temperature destruction of L_1. ×- - - -×, legal standards of pasteurization; ●—●, >99% destruction L_1; ○- - -○, 50% destruction L_1.

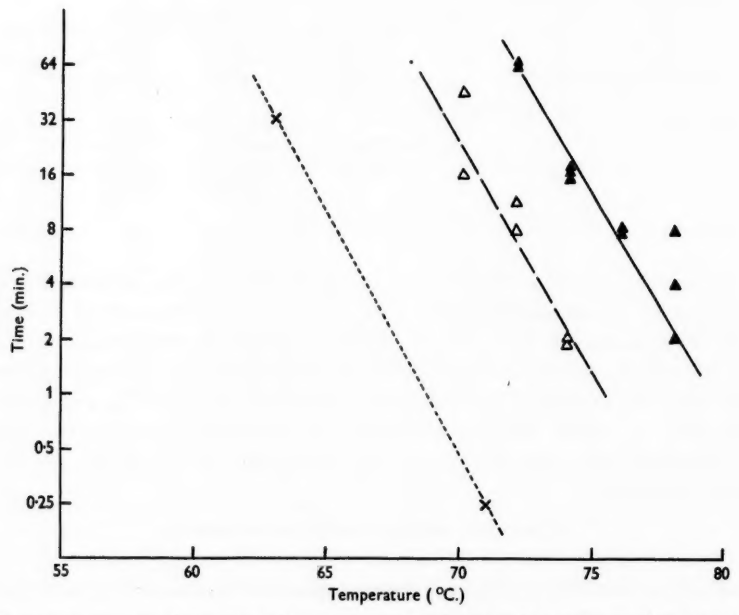

Fig. 2. The time-temperature destruction of L_2. ×- - - -×, legal standards of pasteurization; ▲—▲, >99% destruction L_2; △- - -△, 50% destruction L_2.

A sample of colostrum (C) treated with H_2O_2 and catalase to destroy L_2 (see below, p. 333) was used as a source of L_1. It contained 550 units of L_1 per ml. and no L_2. A sample of bulk milk heated at pH 7·0 and 70° C. for 20 min. (M^{70}) and containing 100 units of L_2 per ml. and no L_1 was used as a source of L_2. These preparations (the colostrum was diluted 1/10) were compared with a sample of bulk milk heated to 60° C. for 20 min. (M^{60}) containing about 100 units L_1 and 100 units L_2 per ml. and with a sample of bulk milk heated to 80° C. for 20 min. (M^{80}), where the two substances were destroyed. The procedure consisted in diluting each of these preparations in powers of 2 with sterilized milk. Each of a series of test-tubes then received 1 ml. of each dilution and 1 ml. of sterilized milk inoculated with 0·1% of an 18 hr. culture of the test organism. After 20 hr. incubation, the tubes were filled with 10 ml. of water saturated with chloroform to stop the growth in the cultures, and the pH determined by the glass electrode.

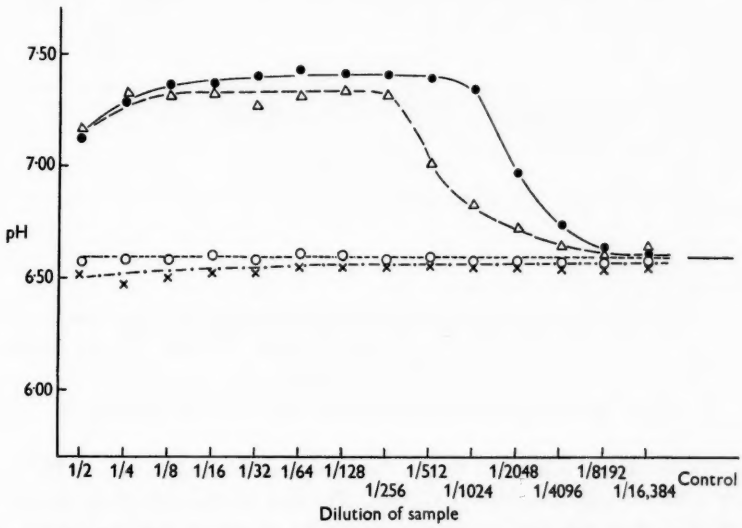

Fig. 3. The sensitivity of *Str. pyogenes* to L_1 and L_2. Dose-response curves obtained with: ●—●, milk heated at 60° C. (L_1+L_2); △- - -△, milk heated at 70° C. (L_2); ○- - -○, colostrum treated by H_2O_2 (L_1); ×·····×, milk heated at 80° C.

Results

Figs. 3–6 show the dose-response curves (dilution against pH) obtained with the following four organisms:

Str. pyogenes 'Richards' stock culture; grown and tested at 37° C.

Str. agalactiae (group I, β-haemolytic streptococcus), a culture freshly isolated from mastitis milk; grown and tested at 37° C.

Staphylococcus aureus (αβ-haemolytic, coagulase positive staphylococcus), a culture freshly isolated from mastitis milk; grown and tested at 37° C.

Str. lactis 'M_1' stock culture; grown and tested at 30° C.

Fig. 3 shows that *Str. pyogenes*, which grows relatively poorly in sterilized milk (milk steamed for 30 min. on three successive days), was strongly inhibited by M^{60} (L_1+L_2) and by M^{70} (L_2) but not by C (L_1).

The response of *Str. pyogenes* to raw milk in a medium containing half milk and half glucose-lemco-broth has already been described (1). The shape of the curve obtained was an indication of the presence of the two substances L_1 and L_2. In sterilized milk containing 0·25% peptone, where the test organism gave a good growth, only the addition of milk containing L_1 and L_2 had an inhibitory effect. It appears, then, that the inhibitory substance L_2, when separated from L_1, requires for its action a deficient medium such as sterilized milk.

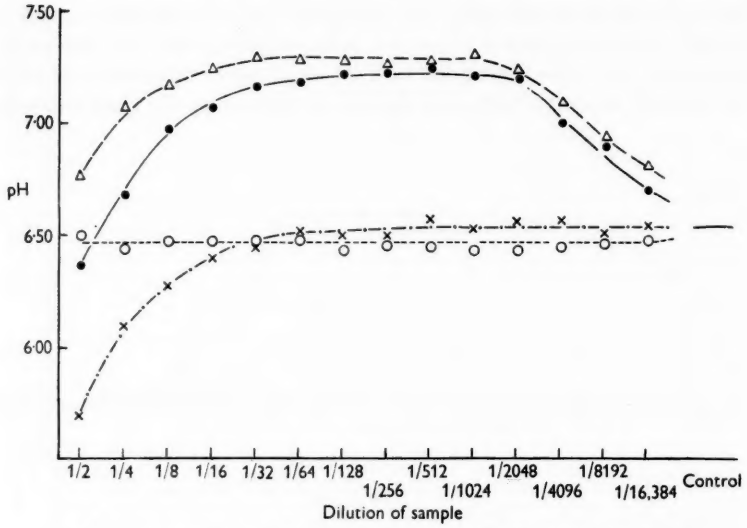

Fig. 4. The sensitivity of *Str. agalactiae* to L_1 and L_2. For legend see Fig. 3.

The responses of *Str. agalactiae* to the four preparations are shown in Fig. 4. M^{60} is inhibitory even at high dilutions (1/16,000). The low acidity which developed in the control showed that the test organism grew only poorly in sterilized milk. At the beginning of the dilution series, the ascending part of the curve indicated that the response was due to a stimulatory substance present in raw milk but not in sterilized milk which enabled the organism to overcome the inhibition. Heating the milk at 80° C. destroys L_1 and L_2 leaving intact the stimulatory substance. This substance improves considerably the growth of the test organism in sterilized milk.

The milk heated at 70° C. where L_1 had been destroyed appeared to be more inhibitory than the milk heated at 60° C. It is also interesting that colostrum, which did not show a stimulatory effect comparable with the effect of M^{80}, slightly improved the growth of the organism at high dilution. The addition of peptone to the diluent improved considerably the growth of *Str. agalactiae* but completely masked the inhibitory effect.

Staph. aureus was inhibited by M^{60} ($L_1 + L_2$), to a smaller extent by M^{70} (L_2) and only slightly by colostrum (L_1). No stimulatory effect was noticeable with M^{80} (Fig. 5).

Fig. 6 represents the inhibition of a strain of *Str. lactis* 'M_1' grown and tested at 30° C. This organism showed good growth in sterilized milk without the addition of peptone. The response curve obtained with M^{60} shows a strong inhibition, but with a stimulatory

effect at the lowest dilutions. With M^{70} the inhibition was weaker and it disappeared completely when the milk was heated at 80° C. The stimulation at the higher concentrations was also only slightly noticeable, the growth being probably limited by the low pH value reached. Colostrum seemed to be without effect.

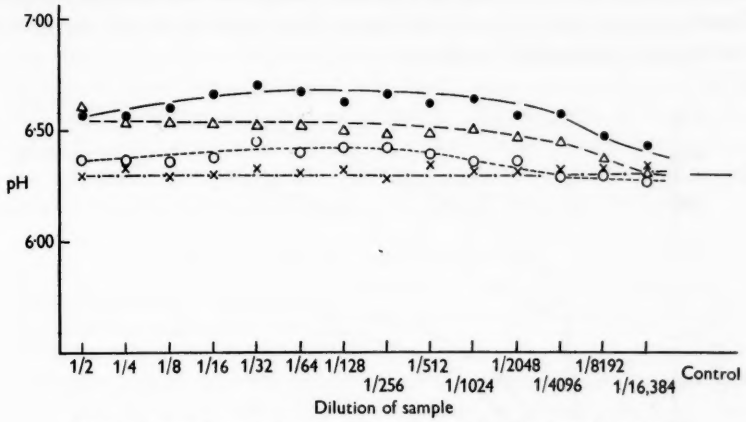

Fig. 5. The sensitivity of *Staph. aureus* to L_1 and L_2. For legend see Fig. 3.

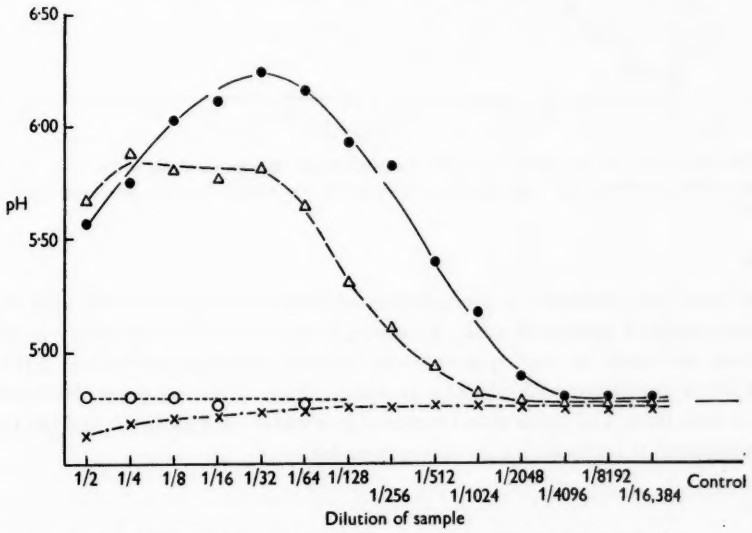

Fig. 6. The sensitivity of *Str. lactis* to L_1 and L_2. For legend see Fig. 3.

The growth of Streptococcus pyogenes *in presence of lactenin*

This organism has been shown to die out rapidly in raw milk (7). However the action of lactenin depends on the medium and the temperature of incubation used. Wilson & Rosenblum (6) observed no decrease of the count of bacteria inoculated into milk at 0° C. At 37° C. the decrease was maximum, but in a medium containing peptone, the organism was able to grow in the presence of raw milk especially if a heavy inoculum was used (6).

In order to investigate more accurately the mode of action of lactenin on *Str. pyogenes*, it was thought that a study of the growth of this organism in the presence of various concentrations of lactenin would be of interest. It is important in particular to know at which stage of the growth of the organism lactenin is active. For this study the method of estimating the growth by measuring the acid production was deemed unsuitable. A turbidimetric method was chosen for measuring the growth of the test organism because of the advantages emphasized by Monod (8).

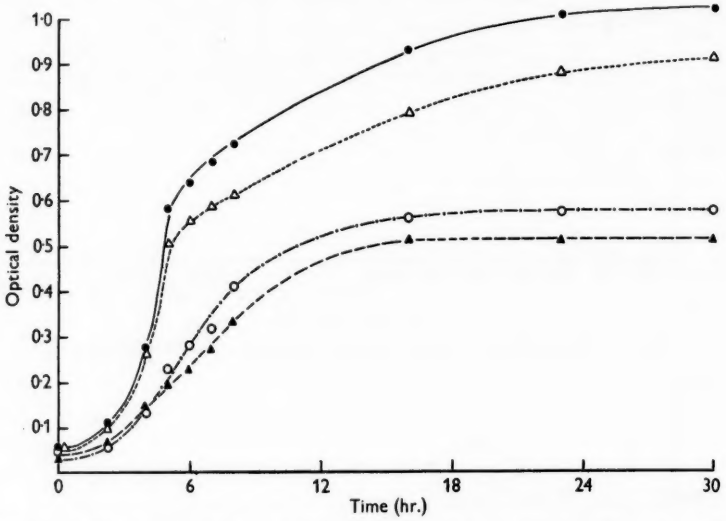

Fig. 7. The growth of *Str. pyogenes* in raw and sterilized whey. ●—●, sterilized whey; △- - -△, sterilized whey + 1/64 raw whey; ▲- - -▲, sterilized whey + 1/16 raw whey; ○····○, sterilized whey + 1/4 raw whey.

Method

Clear whey was prepared by precipitation of casein with hydrochloric acid from raw and from sterilized separated milk. Dilutions in powers of 2 of raw whey in sterilized whey were delivered in 5 ml. quantities in optically standardized tubes. Five ml. of glucose-broth inoculated with 3% of a growing culture of *Str. pyogenes* 'Richards' were added to each tube. The tubes were incubated in a water-bath at 37° C. and the turbidity was determined at intervals by an absorptiometer.

Results

Fig. 7 represents the growth curves obtained by plotting the optical densities (optical density = log. light intensity transmitted by medium minus log. light intensity transmitted by culture) against time, for various concentrations of raw whey. It shows that the test organism was able to grow in all tubes, but the rate of growth obtained with the highest concentrations of raw whey were smaller than that obtained with the lower concentrations and with the control containing only sterilized whey.

It is interesting that lactenin acts on the rate of growth of the organism and not on the lag phase of the growth. It is probable that, when the maximum growth is attained in

the highest concentrations of lactenin, the organisms begin to die out. These results would agree with the observations of other workers (6) who found by counting on agar an increase of the number of organisms inoculated into the milk during the first hours of incubation, followed by a decrease of this number afterwards.

Some other experiments have shown that the number of bacteria present has little influence on the action of lactenin: varying the size of the inoculum 10,000-fold has no effect on the titre obtained in the estimation of lactenin. Moreover, cultures showing no growth after incubation in the presence of lactenin, were revived by diluting the inhibitory substances with suitable volumes of broth. Although the distinction between bactericidal and bacteriostatic substances is difficult to define, these results indicate that lactenin has a bacteriostatic action. The inhibition appeared to correspond to a limited growth of the organism, depending only on the composition of the medium.

Effect of hydrogen peroxide on lactenin

It has been shown (9,1) that lactenin is inactive in anaerobic conditions and in the presence of cysteine. In the following experiments the action of hydrogen peroxide on lactenin has been studied by way of contrast.

When hydrogen peroxide is added to raw milk, the lactenin titre of the milk is reduced (Table 6). If a sample of raw milk is left for some hours in contact with 0·6% H_2O_2 and then catalase added to destroy the remaining H_2O_2 the milk is no longer inhibitory. The

Table 6. *The inhibitory activity of mixtures of raw milk and hydrogen peroxide on the growth of* Str. pyogenes

Dilutions in autoclaved milk	Raw milk	Raw milk + 1/500 6% H_2O_2	Raw milk + 1/2500 6% H_2O_2	Raw milk + 1/12,500 6% H_2O_2	Raw milk + 1/10 6% H_2O_2 and catalase	Autoclaved milk + 1/500 6% H_2O_2
1/6	−	−	−	−	+ + +	+
1/9	−	−	−	−	+ + + +	+ + +
1/13	−	+	−	−	+ + + +	+ + +
1/20	−	+ +	−	−	+ + + +	+ + +
1/30	−	+ + +	−	−	+ + + +	+ + +
1/44	−	+ + +	+	−	+ + + +	+ + + +
1/68	−	+ + +	+ +	−	+ + + +	+ + + +
1/100	−	+ + + +	+ + +	−	+ + + +	+ + + +
1/152	−	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
1/226	+ + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
1/340	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
1/500	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
Control	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +

− = no growth of the test organism.

+ to + + + + = approximate extent of growth of the test organism in milk containing bromocresol purple, corresponding to the following pH values:

− = > 6·5; + = 6·1 to 6·5; + + = 5·7 to 6·1; + + + = 5·3 to 5·7; + + + + = < 5·3.

separate assays of L_1 and L_2 in a sample of milk treated with H_2O_2 and catalase show that L_2 is destroyed while L_1 remains in relatively high concentration. This can be used for separating the two substances in milk or colostrum: for instance a sample of colostrum containing originally 1500 units of L_1 and 20 units of L_2 had after this treatment a content of 600 units of L_1 and no L_2, and was used as a source of L_1 (see above, p. 329).

As reducing conditions abolish the activity of lactenin, it seems probable that a high oxidation-reduction potential is necessary for its action. It is therefore interesting that hydrogen peroxide also abolishes the action of lactenin. However, the mode of action of

these two substances is different: when treated with hydrogen peroxide, L_2 progressively loses its activity; the L_2 titre of milk decreases as the concentration of H_2O_2 in the mixture increases (Table 6). On the contrary, when increasing concentrations of cysteine are added to milk heated to 60° C. and the mixtures assayed by the dilution method, the assay cannot be read directly. The pH responses obtained (Fig. 8) show that the addition of cysteine to milk abolishes the inhibitory action of lactenin at low dilutions without altering the lactenin titre of the sample: the inhibition is noticeable at approximately the same dilution with and without cysteine, except for the highest concentration of cysteine. This means that cysteine does not destroy lactenin but that it only prevents its inhibitory action when the concentration of cysteine is sufficient.

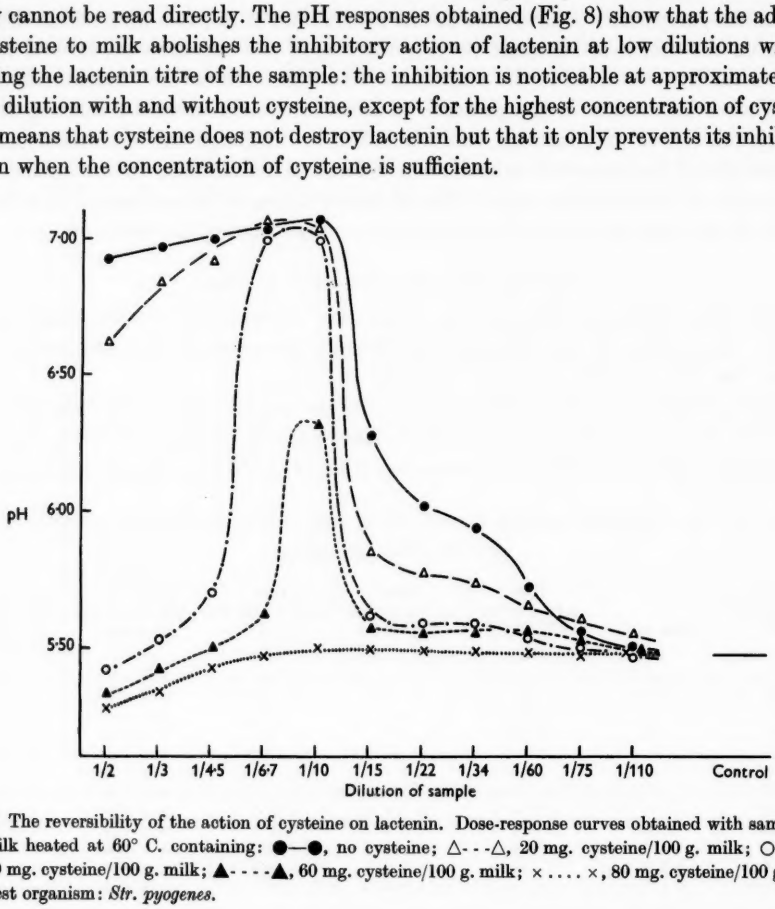

Fig. 8. The reversibility of the action of cysteine on lactenin. Dose-response curves obtained with samples of milk heated at 60° C. containing: ●—●, no cysteine; △- - -△, 20 mg. cysteine/100 g. milk; ○·····○, 40 mg. cysteine/100 g. milk; ▲- · - ·▲, 60 mg. cysteine/100 g. milk; × .... ×, 80 mg. cysteine/100 g. milk. Test organism: *Str. pyogenes*.

DISCUSSION

The results of the estimation of L_1 and L_2 in a number of samples of bulk milk and milk from individual cows showed that the variations observed between samples were not related to the season of the year, the stage of lactation or the age of the cows. The variations were relatively small when compared with those occurring at the time of calving when colostrum contains L_1 in very high proportion.

Cows sensitive to mastitis produced milk with about the same lactenin content as resistant ones, although in a few cases the L_2 content of the former was relatively high. This would appear to confirm the opinion of Wilson & Rosenblum (4) that lactenin plays no role in mastitis. However, lactenin is possibly not the only antibacterial substance of milk. Other bacteriostatic substances may be active *in vivo* against mastitis organisms

and have a role in the development of infection in the udder. Milk also contains some stimulatory substances, interfering with lactenin, and these substances may be responsible for the growth of pathogens in the udder (10).

The various organisms tested have shown different sensitivities to L_1 and L_2 and to the stimulatory substances of milk. For instance, *Str. agalactiae* is very sensitive to lactenin when raw milk is diluted in sterilized milk but not in the same undiluted raw milk, where it is able to grow because of the presence of the stimulatory substances.

After milking lactenin has certainly an important role in the development of micro-organisms in milk. It is probable that in the few hours after milking there is in the milk a selection of micro-organisms resistant to lactenin which develop without hindrance, while sensitive organisms disappear.

The role of lactenin in the keeping quality of pasteurized milk is difficult to ascertain. Ordinary commercial pasteurization does not affect greatly the lactenin content of milk since only L_1 is partly destroyed by the heat treatment corresponding to the legal standards of pasteurization. Under these conditions it is probable that lactenin inhibits the development of the organisms in milk and contributes to its keeping quality. If the heat treatment of milk exceeds markedly these standards, L_1 and even L_2 may be destroyed and the keeping quality be reduced, especially if the milk is contaminated after pasteurization.

The control of the bacterial development in raw milk is specially important in cheese-making. Czulak & Meanwell (11) have shown that the sensitivity of lactic acid bacteria to the inhibition by raw milk is variable, depending on the strains used. They observed a seasonal variation in the inhibitory action of milk and suggested that summer milk may contain a growth factor which enables the sensitive strains to overcome the inhibitory action of milk. Our own results confirm this hypothesis since the dose-response curve obtained with the strain of *Str. lactis* used showed that this organism was sensitive to lactenin but that a stimulatory substance present in raw milk was interfering with the inhibition. The problem is therefore complex. It is probable that lactenin is mainly responsible for the inhibition of lactic acid bacteria in raw milk. However, we have shown that the lactenin content of milk is relatively constant during the year. The variations observed in the development of starters during the year are therefore probably to be accounted for by variations in the stimulatory substances of milk.

SUMMARY

1. The L_1 and L_2 contents of a number of samples of bulk milk and milk from separate quarters of cows with and without infection have been determined. The milk of cows sensitive to mastitis has about the same content as the milk from resistant ones.

2. The effect of heat on L_1 and L_2 has been observed for various combinations of time and temperature. Heat treatment corresponding to the legal standards of pasteurization destroyed 50% L_1, but did not affect L_2.

3. The inhibitory action of L_1 and L_2 has been observed on several micro-organisms. All the organisms tested were sensitive to lactenin in sterilized milk. Stimulatory substances have been shown to interfere with the inhibition by lactenin.

4. The action of lactenin on the growth of *Str. pyogenes* has been studied. The effect of hydrogen peroxide on lactenin has been compared with that of cysteine.

The author wishes to thank Dr A. Hirsch and Dr N. J. Berridge for advice and criticism, and Miss Grace Thorner for technical assistance.

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560. THE INCIDENCE OF PENICILLIN IN MILK SUPPLIES

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(With 1 Figure)

INTRODUCTION

In recent years attention has been directed to the effect on cheese manufacture of the presence of residual penicillin in milk following its veterinary use. The amount of this antibiotic which is excreted at each milking under various conditions has been studied, and the concentration required to interfere with starter activity has been investigated by a number of workers. An excellent review of this work has been made by Overby(1).

There is, however, little corresponding information available on conditions in this country, and the present investigation was undertaken to throw light on the effects of penicillin administration in English herds as regards the extent to which it is present in market milk and its possible effect on factory cheese manufacture.

Two surveys were carried out, one in the summer of 1951 and a second in the winter of 1953, and the results are summarized in the following paper, together with an account of the technique of assay used for the determination of penicillin in large numbers of samples.

EXPERIMENTAL

Source of samples

Most of the samples examined were taken from churns or bulk milks immediately on arrival at the processing depot or cheese factory. Some which were collected at a distance from the laboratory were not examined until 24–48 hr. after collection. Experiments showed that provided the samples were not grossly contaminated with penicillinase-producing bacteria (in practice, coliform organisms) delays up to 48 hr. did not cause any loss if an efficient ice pack was used. Sour samples were discarded.

Assay of penicillin

The penicillin was assayed by a modification of the method of Sherwood(2) with cups instead of paper disks, and using as test organism a strain of *Sarcina lutea* obtained from Australia (A.T.C.C. 9341). Assays were conducted at two levels of concentration of both test and standard, on each of four plates, the thickness of agar carrying test organisms being maintained constant at about 1 mm. Each of the four replicate plates carried four samples—two standards and two unknowns, the ratio of concentrations being 3·16. Both standard and test concentrations were adjusted so that the zone sizes for each were not markedly different, and since with *S. lutea* the relationship log. concentration/zone size departs from linearity above 0·3 unit/ml., both concentrations were adjusted to 0·08 and 0·25 unit/ml. The cups were rested on the surface of the agar, the zones measured after 20 hr. incubation at 30° C., either with calipers or with an epidiascope and the penicillin

content computed by the method of Bliss (3). The lowest amount of penicillin which could be detected by the method used, with reasonable confidence and under optimum conditions was about 0·03 unit/ml. All samples were subjected to the following preliminary sorting test.

Sorting test

Three samples were set up on each plate and incubated at 30° C. overnight with one weak standard containing 0·08 unit/ml., the remainder of the milk being held in the refrigerator meanwhile. If any sample gave a positive result the amount present was then estimated on four plates at two levels as described above, but those samples which contained penicillin in excess of 0·3 unit/ml. were first subjected to a further dilution test. This consisted of making serial dilutions of 1/10th, 1/20th., ..., 1/100th, and mounting in eight cups on a 6 in. plate. Visual inspection after 18 hr. at 30° C. permitted the selection of the appropriate dilution, and the above four plate assay was then carried out.

At the same time as the quantitative test was made each milk was set up on a plate containing one test and one standard, together with duplicates to which one drop of penicillinase had been added. Occasionally samples were encountered giving zones not due to penicillin since they were unaffected by the presence of penicillinase.

RESULTS

(*a*) *First survey, May–September* 1951

In Table 1 are shown the collected results of 1082 examinations of milk entering five plants including two cheese factories.

Table 1. *Incidence of penicillin in raw milk*, 1951

Source of sample	Sampled	No. tested	No. positive	% positive
Rail tank, received at London depots	On arrival	59	0	0·0
Producers' individual churns at London depots	On arrival	712	10	1·4
Bulk vat milk (1000 gal.) at cheese factory, Dorset	On arrival and sent to London in ice pack	132	0	0·0
Producers' individual churns from cheese factory, Shropshire	On arrival and sent to London in ice pack	179	5	2·8

Thirteen of the Shropshire samples gave a very small just detectable zone which was not due to penicillin. These were excluded.

This survey showed that penicillin was absent in bulked milk, but might be present in individual churns in small quantities. Of ten churns giving positive results which were sampled on arrival at the London processing depots, four came from two farms belonging to the same producer, but at widely spaced intervals. Only one had a content exceeding 0·25 unit/ml., the others containing very much smaller amounts.

Of the five samples containing penicillin received at the Shropshire factory, four came from one farm on four consecutive days and had penicillin contents of 15·0, 15·0, 9·0 and 3·0 units/ml. This suggests that the first and second milkings following injection had been sent into the factory. The inclusion of this milk with an 800 gal. bulk led to starter failure, the corresponding whey containing 1 unit/ml. of penicillin.

(*b*) *Second survey, February–March* 1953

This survey was made at a cheese factory in Shropshire, and Table 2 summarizes the results of 557 tests.

This table shows that for farmers' composites the incidence of penicillin-containing milk is of the same order as that found for individual churns in 1951. Eight of the fourteen positive composites contained penicillin in excess of 0·1 unit/ml. This is the level of penicillin in milk at which starter inhibition may be expected, as several investigators have shown (Hunter(4,5,6), Katznelson & Hood(7)), though smaller amounts may affect the growth of some starters. Two of these composite milks contained 0·71 and 1·17 units/ml. respectively, and a 10% admixture of either of these (and particularly the latter) with penicillin-free milk would have rendered the resultant bulk inhibitory.

Table 2. *Incidence of penicillin in raw milk supplies at a Shropshire cheese factory*

Source of sample	Sampled	No. tested	No. positive	% positive
Producers' daily consignments	Composite from each producer	441	14	3·2
Producers' daily consignments, repeat samples from individual churns	Sampled within 3 days following the report of a positive composite	60	18	30·0
Bulk supplies road tanks, 2000–3000 gal.	On arrival	33	0	0·0
Bulk milk for starter culture	Sample taken from vessels after bulking of farmers' churns, before pasteurization	23	2	8·7

Of the individual churns taken from any producers' consignment whose composite gave a positive result the previous day, only five out of the eighteen still giving a positive result contained amounts exceeding 0·1 unit/ml. though one contained 0·78 unit/ml., and this stimulated a brief investigation of the rate of excretion of penicillin from treated animals.

A herd was selected in which about 70% of the cows were diseased and were given three treatments at 3-day intervals of 100,000 units procaine penicillin with aluminium stearate per quarter. Composite samples from each milking were examined,* and the results were plotted (Fig. 1). There is a striking regularity in the amount of antibiotic excreted except for the first milking after each administration.

Our results indicate that all milk up to the fifth milking and particularly that of the first milking after administration of penicillin contains inhibitory concentrations of penicillin. As an example of its importance, in our experiment the first milking after the second administration of penicillin contained 29 units/ml. in 60 gal. Bulking this with 6000 gal. of penicillin-free milk would still give rise to inhibition.

Reverting to Table 2, the two penicillin positive samples of bulk milk for starter culture contained 0·06 and 0·04 unit/ml. which was sufficient to cause slowing up of starter activity. Milk used for starter preparation at this factory normally consists of the bulk of certain selected supplies well mixed and pasteurized before inoculation. In the

* The authors are indebted to Mr A. C. Page of United Dairies (Wholesale) Ltd., Maiden Newton, Dorset, for arranging the collection of samples and data for this part of the work.

cases mentioned the penicillin content was principally derived from a single producer's supply containing 0·3 unit/ml. These cases demonstrate the importance of selecting milk for starter preparation from as large a number of supplies as possible.

To these results may be added those obtained at random during the last 2 years from milks sent in for examination because of slowness experienced in cheese-making. Of twenty samples of bulk milk two gave positive results (0·08 and 0·11 unit/ml.). In addition, seven wheys from slow vats were examined and these gave one positive result. This particular whey contained 0·23 unit/ml. and this constituted a definite case of inhibition due to the presence of penicillin.

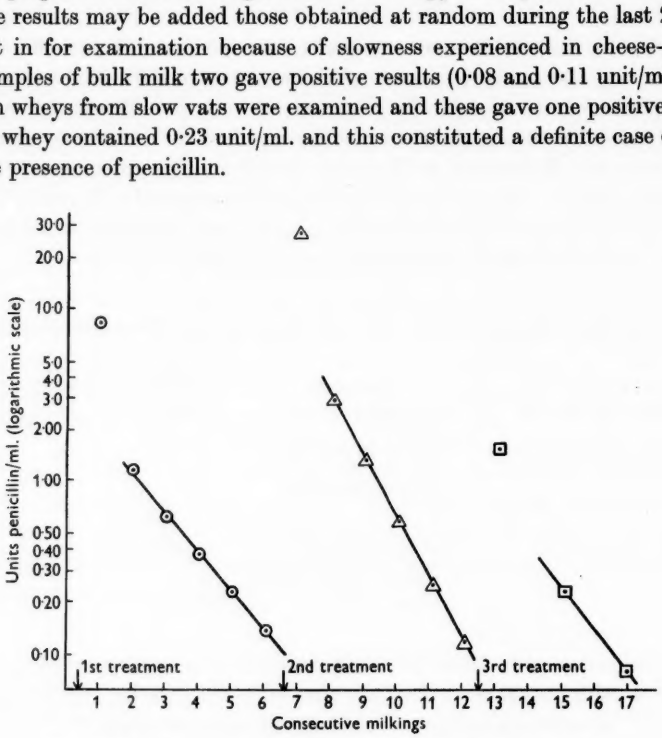

Fig. 1. Rate of excretion of penicillin from a penicillin-treated herd.

DISCUSSION

It has been noted by a number of workers that cows treated with procaine penicillin and aluminium stearate (100,000 units per quarter) produce milk which may contain amounts of penicillin sufficient to inhibit starter activity up to the fifth milking, and this has been confirmed in the present investigation in which in a herd of fifty cows containing thirty-seven treated in this way, amounts of penicillin exceeding 0·1 unit/ml. could be obtained up to the fifth milking. In general, milk from subsequent milkings would be expected to give no trouble in the vats provided it was mixed with penicillin-free bulk at least in the proportion of one to four.

In spite of these findings, whilst the incidence of penicillin-containing milk remains of the order of 3% in churn milk supplies, there does not appear to be (as yet) any serious problem to be expected in factory cheese-making from the veterinary use of this antibiotic provided supplies are adequately mixed.

On the other hand, it has been shown that trouble can arise from this cause and it may well be that the veterinary use of penicillin and other antibiotics will increase to a point where serious embarrassment to cheese-making would arise.

Administrative machinery is therefore needed whereby buyers may receive immediate notification that antibiotic treatment is being applied to herds, and arrangements made for the segregation of such milk from the normal intake of cheese-making establishments.

SUMMARY

1. In surveys carried out in the summer of 1951, 1·4% of churn milk contained penicillin in one group of tests and 2·8% in another. Bulk milk was free from this antibiotic.

2. In a further survey carried out in the winter of 1953, 3·2% of composite churn milk contained penicillin. Bulk milk was again free, but small aggregates of a few supplies used for making bulk starters sometimes contained penicillin.

3. Of twenty samples of milk and seven of whey examined during 1951–3, and which were implicated in starter failure, only two milks and one whey contained penicillin in significant amounts.

4. The rate of excretion of penicillin from the composite milk of a herd containing thirty-seven treated cows was determined. Milk up to the fifth milking contained sufficient penicillin to cause starter inhibition.

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561. THE USE OF PHAGE TYPING AND PENICILLIN SENSITIVITY TESTS IN STUDIES OF STAPHYLOCOCCI FROM BOVINE MASTITIS

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INTRODUCTION

The present study of *Staphylococcus aureus* in bovine mastitis was undertaken to examine the value of phage typing, antibiotic sensitivity, and haemolysis on blood plates as aids in distinguishing new infections from relapses. It was made in several dairy herds that had been under bacteriological observation for a number of years and in which the incidence of staphylococcus infections of the udder was known. In one herd, 'N', a special study was made of the staphylococci from all manifest infections before and after treatment with antibiotics. The use of differences in haemolysis for distinguishing strains will be referred to only briefly in this paper.

METHODS

Isolation of staphylococci. About 0·05 ml. of the milk was smeared over the surface of a thin aesculin sheep-blood (7%) agar plate (1). After 48 hr. incubation in air at 37° C. the number of staphylococcus colonies was estimated. Colonies exhibiting zones typical of β lysin were regarded as *Staph. aureus* without further tests for identification. Colonies that gave complete haemolysis, or were non-haemolytic, were distinguished from micrococci, i.e. coagulase-negative cocci, by their colour and by the slide-coagulase test. When the result of the latter was doubtful, the test was repeated using different plasmas and the no. 2 tube coagulase test of Williams & Harper (2).

Throughout this paper we use the terms *Staph. aureus* and 'staphylococcus' as synonyms for 'coagulase-positive staphylococci'.

Examination of staphylococci. For most herds except N, a single colony was selected for further examination, unless different colonies showed different types of haemolysis, when a representative of each was picked.

In herd N, two or more colonies of staphylococci were nearly always examined, and with samples taken at the time of treatment, four colonies were selected. In addition, fourteen infected quarters were studied over 4–19 months; from each of ten of these, ten colonies were taken at random from the first sample, and three or four colonies from each subsequent sample.

Colonies selected for further examination were streaked on 5% washed sheep-blood agar plates and thence a single isolated colony was picked and tested for coagulase, phage type, haemolytic properties and sensitivity to antibiotics.

Infections. The bacteriological condition of the udder was determined by examining fresh foremilk samples collected aseptically from single quarters (1).

An intramammary infection was assumed to be present if (*a*) *Staph. aureus* was present

in large numbers and there were clinical signs of disease or (*b*) staphylococci were present in large numbers, e.g. 1000 per ml. or more, in three out of four samples, provided that teat-apex abrasions were absent. In the presence of such abrasions a high count of staphylococci was taken to indicate intrammary infection only if the Whiteside and electrical conductivity tests were positive (1).

Treatment. Treatment with antibiotics was generally given only for manifest infections. The results of treatment will be discussed in another publication, but successive courses of treatment were often necessary before the staphylococci were eliminated. The longest course of treatment consisted of five infusions, each of either 100,000 units of penicillin or 250,000 units of dihydrostreptomycin, in a period of 8 days.

An infected quarter was assumed to be cured if *Staph. aureus* was not found in any of four samples taken at weekly intervals after treatment.

Phage typing. The methods of type identification were those described by Williams & Rippon (3,4). In many cases the staphylococci had rather complex phage-susceptibility patterns, and it was not always possible to be certain when two given strains belonged to the same type. However, it was possible to recognize twelve fairly characteristic patterns into which most of the strains fell. These patterns are referred to in the present report under the code letters A to M, as given below. It should be pointed out that not all the strains entered under a given letter had an identical phage pattern, but, relying largely on the experience quoted by Williams & Rippon (3), it was considered that they could all have had a common source.

Code letter	Phage susceptibility pattern determined with phages at routine test dilution
A	42D, 7/42D/44/44A, and other combinations of these four phages
B	Numerous phages of groups I and III
C	Lysed by one or more phages of group I and also by 42B
D	7+ or 7/52A+
E	3C/7/29/42B/52/52A/54 (and 7/52/54+)
F	7/42B/54+
H	47A
I	42C/42D/44/44A/54+
J	54+
K	6/47/53/54+
L	6/7/47/53/54+
M	53/54
NR	No reaction } some of which may or may not be the same as A
VS	Very sensitive } some of which may or may not be the same as A

Haemolysis. The type of haemolysis was first assessed on washed sheep and rabbit blood agar and confirmed by testing for the inhibition of haemolysis by antitoxin incorporated in a strip of agar or filter-paper (5,6).

Sensitivity to antibiotics

Routine cartwheel method. One drop, about 0·03 g., of the antibiotic preparation, as sold for intramammary infusion, was expressed from the collapsible tube on to the centre of a washed sheep blood agar plate, which was then held for the following times to allow for diffusion of the antibiotic:

Antibiotic preparation	Time for diffusion (hr.)
Penicillin: about 125,000 units/g. (Burroughs Wellcome and Co.)	0–2
Dihydrostreptomycin: about 100,000 units/g. (Glaxo Laboratories Ltd.)	18–24
'Protegan' about 40 mg. sulphathiazole and 160 mg. dibromopropamidine per g. (May and Baker Ltd.)	40–48
Nisin, 0·125 g./ml. (Fisons Ltd.)	40–48

Ten to twelve strains, including the Oxford staphylococcus (NCTC 6571), grown overnight in Lemco broth, were then streaked in succession round the plate, from the edge to within 2–3 mm. of the central drop of the antibiotic. After incubation at 37° C. for 24 hr. a record was made of the degree of inhibition and whether or not growth terminated gradually or abruptly.

A strain was considered to be sensitive if it was inhibited to about the same extent as the control Oxford staphylococcus, and resistant if the zone of inhibition was only half that of the control. On the plates with penicillin these zones were 18–24 and 2–8 mm. respectively.

Broth method. Serial dilutions in broth of penicillin G were made to determine the concentrations inhibiting the growth of representative strains. The inoculum per 10 ml. was 0·07 ml. of an 18 hr. broth culture.

Spot plate method. Twofold dilutions of streptomycin sulphate were made in nutrient agar at pH 8 and the plates spotted with representative strains.

Penicillinase. The spot plate method of Bondi & Dietz(7) and of Gots(8) was used, with 0·05 unit/ml. of penicillin and undiluted broth cultures. After 48 hr. incubation, growth of the Oxford staphylococcus under or around the test organisms was regarded as indicative of penicillinase production.

RESULTS

Occurrence of different phage types of staphylococci in one herd

Staphylococci were obtained from milk samples from 186 cows in fifteen herds (Table 1). Three herds (D, 5 and N) had a relatively high prevalence of staphylococcus infection (> 50%) and staphylococci with phage patterns A and C were common in all three herds. The 'eight other herds' were more lightly infected, and only a proportion of the strains from infected cows were typed; they were mainly of phage pattern A, but seven other types were represented. From twenty of thirty-one clinical cases in four additional herds, staphylococci of phage pattern B, C and D were isolated.

Thus, in heavily infected herds at least, infections were due to more than one phage-type. Over the whole study staphylococci with pattern A, which includes the 42D type regarded as typical of bovine strains(9,10) was the commonest, but pattern C, with lysis by phage 42B and one or more of the phages 29, 44, 52 or 52A, was nearly as common.

Occurrence of two phage types in single samples from one quarter

Altogether thirteen of 104 quarters studied, mainly in herd N, contained two types of staphylococci, but with one exception two types were isolated only when abrasions were present at the teat orifice (Table 2). Whereas such double infection was found in only 2·5% of quarters with healthy teats, it was present in 6·7% of quarters with a teat erosion, and as many as 50% of eighteen quarters with a small sore at the teat orifice.

In fourteen of the seventeen samples (Table 2) containing two phage types, the two types differed distinctly in haemolytic properties, but in only five of the fourteen cases was one type sensitive and the other resistant to penicillin. The staphylococci from three samples showed no apparent difference in haemolytic properties but one type was penicillin sensitive and the other resistant.

When two phage types of staphylococci with differing haemolytic properties were found in a quarter the colonies of one type were, with one exception, few in number on the

Table 1. *The phage patterns of staphylococci recovered from individual quarter milk samples in* 1950–1

Farm	No. of cows examined	No. of quarters infected with staphylococci	No. of quarters yielding staphylococci of the following phage patterns*															No. of quarters diseased when sampled
			A	B	C	D	E	F	H	I	J	K	L	M	Others	NR	VS	
D	58	57	14	9	13	10	—	1	1	—	—	—	—	—	—	2	7	0
5	68	66	22	21	9	—	—	—	—	—	—	1	—	—	—	5	8	0
N	81	89	21	—	41	4	1	1	—	—	—	—	12	6	1	1	1	40
Eight other herds	—	33	18	—	—	3	—	—	—	1	1	1	—	—	2	7	—	4
Four other herds; samples from diseased quarters only	—	31	2	5	9	6	1	—	—	—	—	—	—	—	—	6	2	31
Total quarters	—	276	77	35	72	23	2	2	1	1	1	2	12	6	3	21	18	75
Total colonies phage typed	—	896	183	36	339	37	2	2	1	1	1	2	181	61	6	26	18	—

* The code letters correspond to phage patterns given in the text.

Table 2. *Frequency of two phage types in single quarters*

State of teat orifice	No. of colonies from each sample	No. of milk samples	Samples with two phage types		Quarters		
						Quarters with two phage types	
			No.	%	No. of quarters	No.	%
Healthy	2	55	0	0	22	0	0
	3–4	27	1	3·7	10	1	10
	5–10	19	0	0	9	0	0
	Sub-total	101	1	1·0	41	1	2·5
Eroded	2	34	5	14·7*	26	3	11·5
	3–4	33	0	0	8	0	0
	5–10	14	0	0	11	0	0
	Sub-total	81	5	6·2	45	3	6·7
Sore present	2	14	8	57	12	6	50
	3–4	3	1	33·3	2	1	50
	5–10	4	2	50	4	2	50
	Sub-total	21	11	52·4	18	9	50·0
	Total	203	17	8·4	104	13	12·5

* The reason for the preponderance of two types in samples from which two colonies only were picked may be the rather more severe teat erosions found in this group.

blood plate and may have been derived directly from infected abrasions at the teat orifice. In one case, the two types (patterns A and D) were probably of intramammary origin for, although the colonies from only two samples were phage typed, five successive samples contained large numbers of the two kinds of haemolytic colony and two of these samples were taken when the teat orifice was normal.

Occurrence of two phage types in successive samples from one quarter

Thirty-five of the 104 quarters were sampled on two or three different occasions at monthly intervals and one colony of *Staph. aureus* from each sample was typed. In all cases, all the staphylococci from each quarter had one phage pattern.

Another fourteen quarters were studied over 4–19 months; only four of eighty-nine samples contained staphylococci of two different phage types and these four samples were from three different quarters.

Occurrence of different types of staphylococci in the separate quarters of an udder

In the three herds D, 5 and N, there were thirty-seven cows each with two infected quarters; of these only seventeen had staphylococci of the same phage type in both quarters.

A further twenty-one cows were infected in three or four quarters and, although twenty had two quarters infected with one type, only five of them had the same phage type in all quarters. Haemolysis was not a good indication of different phage types since in twenty-two cases pairs of strains showed no difference in haemolysis but were different in phage type, and in five cases haemolytic properties differed but not phage type.

New infections and phage type

In herd N the number of new staphylococcus infections developing each month was determined for cows already infected in one or more quarters, and for cows not infected in any quarter at the beginning of the month (Table 3).

Table 3. *New infections and phage type*

	New infections each month							Total new infections	Mean no. of cows and quarters in lactation each month		New infections in dry cows
	Apr.	May	June	July	Aug.	Sept.	Oct.		Cows	Quarters*	
No. of non-infected cows	50	50	38	34	40	49	52	—	43	172	—
A. New infections in these cows	1	4	0	5	1	2	4	17	—	—	9
No. of cows already infected in one or more quarters	23	25	19	17	18	16	20	—	20	50	—
B. New infections in the uninfected quarters of these cows:											
1. Became infected with a phage type already present in another quarter	1	4	0	1	0	1	0	7	—	—	3
2. Became infected with a phage type not present in another quarter	1	4	2	2	2	1	1	13	—	—	3
3. Total new infections in infected cows i.e. 1+2	2	8	2	3	2	2	1	20	—	—	6
Total no. of cows becoming infected with a new phage type of staphylococcus, i.e. $A+B_2$	2	8	2	7	3	3	5	30	63	222	12
Total new infections, i.e. $A+B_3$	3	12	2	8	3	4	5	37	63	222	15

* Quarters not infected with staphylococci at the beginning of each month.

During 7 months there were thirty-seven new infections, twenty in quarters of udders already infected in one quarter, and seventeen in udders previously free from infection. Only seven (19%) of the thirty-seven infections were caused by staphylococci of the phage type already present in the udder. Similarly, only three (20%) of fifteen infections developing in dry cows were due to a staphylococcus of the same phage type as that already present in the udder.

From an inspection of the results it appears that cows already infected were more than twice as likely to acquire new infections as non-infected cows.

Spontaneous changes

Forty infected but untreated quarters were examined on three to ten occasions. In only two did the staphylococci show any change in phage type and in both cases, also, they altered in haemolysis and sensitivity to penicillin. Both changes occurred while the teat apex was unhealthy. However six of the forty quarters remained infected for more than a year without any change in the staphylococci, even though four of them frequently had small erosions at the teat orifice.

Changes following treatment

Staphylococci were considered to have 'persisted' through a course of treatment of a quarter if they were found in milk samples taken within 4 weeks of the end of treatment. In thirty quarters the staphylococci 'persisted' after 1–8 separate courses of treatment, comprising in all twenty courses of penicillin, nineteen of dihydrostreptomycin, twelve of nisin and eleven of Protegan.

The staphylococci persisting after fifty-five of sixty-two courses of treatment showed no significant differences in phage type, penicillin and nisin sensitivity, or haemolysis, from those infecting the quarter before treatment. In one case they differed from the original strain in haemolysis only. In six cases there was a change in phage type and drug sensitivity, which presumably indicates re-infection of the quarter after successful treatment. In only four of the six did the pre- and post-treatment staphylococci differ in haemolytic properties.

With one exception the changes of penicillin sensitivity were from sensitive to resistant. Two of them occurred in udders treated with dihydrostreptomycin. Most of the infections caused by penicillin- and nisin-resistant strains occurred in quarters that had not been treated.

Eighteen of the thirty quarters showed clinical signs before one or other of the unsuccessful treatments, but the staphylococci recovered before and after treatment were of the same phage type.

Phage typing as an aid in assessing cures

According to our definition fifty-five infected quarters were cured by treatment. However, the staphylococci recovered from six quarters in the first 4 weeks after treatment had a different phage pattern from those recovered before treatment and may be regarded as reinfections. Thus the total number of quarters 'cured' was 61. However, eight of the sixty-one became reinfected between 6 and 10 weeks after treatment, in six cases with the type present before treatment. Thus only forty-seven of the sixty-one were still free from staphylococci 10 weeks after treatment.

Severity of infections due to different phage types

In a period of 8 months, thirty-five of seventy-four infected quarters in herd N were clinically diseased (excluding five which showed pus in the strip cup once only). The percentage of quarters showing disease varied from 28·6 with infections caused by staphylococci of phage pattern A to 83·4 with those caused by staphylococci with pattern M (Table 4). It is not, however, possible to take these rates at their face value, since there were various complicating factors. Thus of the eleven infections with phage pattern L,

Table 4. *The severity of infections caused by staphylococci of different phage types*

	A	C	D	L	M	Others	Total
No. of infections	14	37	3	11	6	3	74
No. classed as severe	0	1	0	0	0	0	1
No. classed as moderate	0	5	0	0	1	0	6
No. classed as mild	1	6	1	2	4	0	14
No. classed as trivial	3	5	1	5	0	0	14
Total no. of quarters with manifest disease	4	17	2	7	5	0	35
Percentage of infected quarters with disease	28·6	46	67	63·7	83·4	0	47·3

three were mixed staphylococcus and streptococcus infections and all three produced disease. There were only two other such mixed infections among all the other staphylococcus types. This is probably sufficient to account for the high disease rate in infections with pattern L. Similarly, four of the six infections with pattern M staphylococci became manifest early in lactation, when the disease is known to be most severe.

Eleven of the seventy-four infections disappeared spontaneously after 1–12 months' duration, but there was no apparent association with phage type.

Sensitivity to antibiotics

Sensitivity to penicillin. A total of 1410 colonies of staphylococci from milk samples were tested for sensitivity to penicillin by the cartwheel method, and 282 of them were classed as resistant (Table 5). One hundred and nine of the 1410 strains were tested also by the broth method; seventy-two that had been classed as resistant grew in broth containing 1–400 units/ml. (usually 10–100 units/ml.) and thirty-seven strains that had been classed as sensitive were inhibited by 0·05–0·005 unit/ml. in broth. The growth of resistant strains on the cartwheel plate terminated abruptly and all these strains produced penicillinase.

Sensitivity to dihydrostreptomycin. This antibiotic had been used since 1946 for the treatment of mastitis on four of the farms shown in Table 5.

Of the 1101 colonies tested by the cartwheel method all but ten were, within the limits of the test, as sensitive as the Oxford staphylococcus. These ten colonies were from two quarters, one of which had been treated with the antibiotic. They were inhibited by 6 units of streptomycin, whereas twenty sensitive strains tested at the same time were inhibited by $<0{\cdot}5$–2 units/ml.

Sensitivity to nisin. As previously reported (11) there was complete agreement between resistance and sensitivity to penicillin and nisin.

Sensitivity to 'Protegan'. All of the 658 strains examined were as sensitive as the Oxford staphylococcus to this product.

Table 5. *The penicillin sensitivity of staphylococci from 739 quarters*

Date	Herd	No. of cows examined	Staphylococci recovered from		No. of colonies examined	Approx. annual no. of infections of all types treated with penicillin	Percentage of quarters yielding penicillin-resistant colonies
			Cows	Quarters			
iii. 50	3	61	36	36	36	11	0
ix. 50	3	63	29	49	49	—	0
v. 50	5	62	40	62	62	12	0
ix. 50	5	68	38	66	67	—	0
ix. 50	E	57	27	41	41	47	2·4
vii. 51	E	56	21	29	29	15	0
xi. 50	D	70	31	57	58	12	0
vii. 51	D	85	30	31	31	—	0
xi. 50	4	55	11	22	22	9	0
xi. 50	F	60	22	39	39	14	2·6
viii. 51	Bi	57	1	1	2	10	100
1951	Ba	30	17	23	44	200	83
1951	Cr	40	15	21	39	69	9·5
1949–51	N	140	64	138	752	80	18
1950	Da	50	8	15	15	15	0
1950	SK	45	3	6	6	12	0
1950	L	40	2	2	2	10	0
Clinical cases in the first six herds			82	101	116	—	2
Total			477	739	1410	—	6·8

Resistant strains and the amount of penicillin used

Penicillin-resistant strains were recovered from six of thirteen herds. The three herds, Ba, Cr and N, as shown in Table 5, receiving the most treatment with penicillin were among the four with the highest proportion of resistant staphylococci. Eight months before this study penicillin cream had been applied to the teats of cows in herd Ba for 2 weeks.

Although this report refers mainly to material collected up to the end of 1951, observations have continued. A decrease in the proportion of infections caused by penicillin-resistant staphylococci has been observed in one herd and an increase in others. Coagulase-negative cocci which produced penicillinase have been recovered from five quarters in herds Ba and Cr.

Penicillin sensitivity and phage type

There was a fairly clear relation between phage type and penicillin sensitivity. Thus all the 412 strains with phage pattern B, C, or D from seven herds were sensitive to penicillin, while all of 242 strains with patterns L or M were resistant.

In any herd the penicillin-sensitive and -resistant strains usually had different phage patterns (Table 6); the only exception to this was that some of the staphylococci with pattern A were resistant and some sensitive. However, as Williams Smith showed (12) strains lysed by phage 42D (and therefore included in our pattern A) can be distinguished by the use of more phages; it may therefore be that more than one type is included in our phage pattern A.

As was to be expected from the analysis reported above, infection of one quarter with both sensitive and resistant strains was very rare. In fact, in only one case has a quarter been shown conclusively to be so infected, according to the definition on page 342. This was a quarter in herd Cr, from which both sensitive and resistant strains were isolated from four successive samples. All had the same phage pattern, 42D. In four other

instances sensitive and resistant strains were isolated from a single quarter on one occasion, but for various reasons confirmation of double infection could not be obtained. Transient contamination leading to the apparent presence of sensitive and resistant strains was seen on a few occasions in herd N.

In herd Bi, of 228 quarters examined, one only was found infected, and this with a penicillin-resistant strain of phage pattern A. The quarter had not been treated.

Table 6. *The phage pattern of penicillin resistant and sensitive strains of staphylococci from the same herd*

Herd	No. of quarters yielding resistant strains which were phage typed	No. of colonies of resistant strains which were phage typed	The phage pattern* of strains and the no. of quarters yielding each type	
			Resistant strains	Sensitive strains
E	1	1	VS 1	A 5 D 2 J 1
F	1	1	M 1	Not tested
Bi	1	2	A 1	No other infections
Cr†	6	11	A 4 NR 1 Unclassified 1	A 12 J 1 NR 2
Ba†	27	35	A 20 K 6 E 1	A 12 B 1 —
N†	21	244	L 13 M 6 E 1 VS 1	A 21 C 41 D 4 F 1 NR 1 VS 1 Unclassified 1

* The code letters correspond to phage patterns given in the text.
† Includes strains isolated since those reported in Table 5.

DISCUSSION

It is clear that more than one distinguishable type of staphylococcus is rarely found in established udder infections even when four or five phage types are present in a herd. Although the proportion of quarters yielding strains of two phage types (13%) was similar to that found by Williams Smith (10) only one quarter (1%) could be regarded as more than temporarily infected with two types.

In many herds very small lesions or proliferations at the teat apex are common. They are often heavily infected with staphylococci and even when the end of the teat is thoroughly cleaned and disinfected with tincture of iodine, some contamination of milk samples during collection is unavoidable. When these lesions or erosions are present it is often difficult to establish whether or not there is an intramammary infection without physical and chemical tests. As teats are very exposed to contamination and cross-infection, and minor lesions are usually infected with staphylococci, samples of milk from such cows are likely to contain two phage types of staphylococci.

Although double infection of a single quarter is rare, animals with two quarters infected commonly yield different types of staphylococci from the two infections. Furthermore, of twenty new infections developing in cows already infected in at least one quarter, only seven were with staphylococci of the same type as those already present in the udder.

The udder thus seems less likely to be infected from an adjacent quarter than from an outside source—presumably another udder; the most likely vehicles are the milking machine, the milkers' hands, and the cloth used for washing the udder.

The spontaneous replacement in the udder of one phage type by another was rare and was not associated with the stage of lactation or the appearance of manifest disease.

The assessment of cure in udder infections is based on the absence of the organism for an arbitrarily fixed time. In this study a treatment was assumed to have failed if staphylococci were recovered within 4 weeks. There were sixty-two courses of treatment which failed by this criterion, but phage typing showed that the staphylococci recovered after six of them were of a different type from those present before treatment, and thus in these six cases a re-infection had occurred within 4 weeks of treatment. Other re-infections may have occurred but remained undetected because the staphylococci present before and after treatment were of the same type.

Penicillinase-producing strains were more numerous than penicillin-sensitive strains in only one herd. This herd was heavily infected with *Streptococcus agalactiae* and had had 6 times as much penicillin treatment as any other herd studied.

There was no clear indication that ineffective treatment with penicillin gave rise directly to resistant strains. For example, of seven quarters in herd Ba infected with penicillin-sensitive strains (pattern A) none yielded resistant strains after one to five abortive treatments with penicillin though two of them became infected eventually with resistant strains (pattern A) after being free from staphylococci for 4–6 weeks.

Phage typing is clearly of great value in a study of this sort and by its use we have been able to establish several points in the etiology of staphylococcus mastitis. In many cases, however, the phage susceptibility patterns observed were complex and their interpretation was often so difficult that one would hesitate to recommend the use of the method for a further similar investigation with the present set of phages. It has to be remembered that the phages in use have been selected for their value in identifying staphylococci of human origin, and it is probable that a more satisfactory set could be collected for use on bovine strains.

SUMMARY

In three heavily infected herds there were sufficient types of staphylococci to enable phage typing to be of use in distinguishing between strains present before and after intramammary infusions with antibiotics. Two phage types were rarely found in established infections in one quarter even when three or four distinguishable types were present in a herd. But two types were sometimes found in milk samples when the teat apex was unhealthy and truly aseptic sampling by the usual procedure was impractical. In quarters infected for many months phage type and penicillin sensitivity (and resistance) were found to be very stable. There were only two spontaneous replacements of one phage type by another.

We wish to acknowledge the assistance of Mrs A. Marsden, Miss B. Morgan and Mr J. M. Higgs.

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562. THE STEAM STRIPPING OF TAINTS FROM LIQUIDS

I. AN ANALYSIS OF PROCESSING METHODS WITH PARTICULAR REFERENCE TO THE VACREATOR

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(With 11 Figures)

INTRODUCTION

The cream or milk supplied to butter factories usually contains minute quantities of substances which impart a taste and a smell to the cream. The effects of such tainting substances are very noticeable in the final butter since the mellow flavour of a sweet cream butter does little towards masking the foreign taste of a tainting substance.

In the buttermaking practice of some countries where tainting substances are prevalent (very often derived from the type of feed, or extraneous weeds (1, 11) eaten by the cow) it is usual to reduce the concentrations of the reasonably volatile substances by blowing steam through the cream, by allowing the formation of flash steam, or by boiling the cream. A portion of the taint in vaporous form passes into the steam which is then condensed and rejected, the amount of steam being used depending on the degree of taint removal desired and the vapour pressure of the taint over the cream. The steam can strip any number of taints from the cream simultaneously, but the intensity of treatment is governed by some taint difficult to remove and objectionable to taste.

Certain feed taints have not been removable by the process of steam stripping, probably the most infamous being that due to land cress (*Coronopus didymus*). The tainting substance appears to form (from a mother substance) at the temperature of pasteurization of the cream, and a definite time lag is necessary to complete the reaction so that the cream cannot be made taint-free.

A piece of equipment widely used in New Zealand, Australia, Canada, South Africa and the United States of America is the Vacreator (5, 7, 11) (see Fig. 5). In the Vacreator the cream is formed into drops, mixed with steam, separated in a cyclonic separator and vacuum cooled to about 120° F. Both pasteurization and taint removal are accomplished in the one piece of apparatus.

The steam used, per ton of butter, for the taint-removing process has increased in recent years, and it has become important to reduce the cost and amount of steam used in buttermaking practice.

As steps towards this, the cost of steam generation can be reduced, waste heat can be recovered (8) and the deodorization process can be made more efficient.

It is convenient to approach the problem of improved efficiency in the deodorization of cream by way of a theoretical analysis and as no paper had previously been published on this particular subject the present work was commenced. The study was divided into three stages. First, an analysis which could be applied to existing equipment; secondly, methods which would improve the efficiency; and thirdly, a study of the manner and

rate of taint removal so that equipment of suitable size and type can be designed for the required degree of taint removal.

This present paper deals with the first section of the work and is based on a material balance for each system considered. Combination of the material balance equation with the relation between the taint concentrations in the outgoing vapour and liquid streams gives a value for the final taint concentration.

If the taint transfer process comes to equilibrium, then the relation between the outgoing concentrations is that of the equilibrium characteristic for the taint. In any practical apparatus for the mixing of steam and cream, complete theoretical equilibrium of the taint transfer process is not possible, but it can be approached sufficiently closely to assume equilibrium without introducing appreciable error. For a reference substance in water equilibrium is approached very closely in the Vacreator, so that the analysis presented in this paper and based on the equilibrium characteristic can be used for Vacreator calculations involving the removal of a solute from an aqueous solution.

The conditions for removal of a volatile component from a two-phase system such as cream are more complex than for a simple aqueous solution. Neglecting the particles in fine suspension we can consider that cream consists of a fat phase dispersed as small globules (or clusters of globules) in an aqueous serum. An amount of a third substance completely soluble in fat, or water, or both fat and water will distribute itself between the relative phases according to the distribution laws and will give rise to a particular partial pressure above the cream.

If, at all times in the steam stripping of a solute from cream, the movement of the solute in each of the phases is sufficiently rapid to maintain equilibrium, the computations are similar to those for a solute in a single phase system, and the design of deodorizing plant could be similar in both cases.

The other extreme is the case where the taint held in the fat remains substantially unchanged during the time of contact with the steam. The steam to liquid ratio for deodorization calculations would then be based on the serum quantity and the taint concentration in the serum. Subsequent holding for some time would allow movement of the solute from the fat into the serum until a second equilibrium was reached. It seems possible that this limiting case may have some application to the practical problem since on the assumption of a diffusion rate of 1×10^{-5} sq.ft./hr. for a solute in a fat globule 10μ diameter, 90% reduction of taint would take place in 80–100 sec. provided the globule surface was kept at zero concentration. The required time of contact for a given degree of taint-removal is approximately inversely proportional to the square of the diffusion rate and it would be important to operate a continuous deodorizing process under conditions which will give as high a diffusion rate in the fat as possible.

From a buttermaking point of view it is the concentration of taint in the fat that is important. Possibly, for the very small-sized fat globules, the first case given above, where equilibrium between the dispersed fat phase and the serum is constantly maintained, occurs in practice; for large fat clusters possibly the serum is deodorized initially, the concentration of taint in the fat phase being reduced by subsequent extraction by the serum after the cream has passed from the deodorizer outlet. Finally one must consider a process of taint removal intermediate between these two extremes.

In addition to the processes just outlined a further possibility is the direct action of the

steam on the fat surface, but here again if taint movement within the fat is slow, the contribution made by this operation will be small.

Thus in spite of an incomplete understanding of the mechanism of taint transfer from the fat to the steam, the principles underlying the operation and design of equipment for taint-removal from cream by steam treatment can be developed by assuming equilibrium conditions either of the cream as a whole or of the serum. The following analysis is based on stage equilibrium being reached in one or more vessels.

EQUILIBRIUM CURVES FOR DILUTE SOLUTIONS

The tainting substances in cream are in very minute quantities. The nature of the substances, their relative solubility in fat and water and their boiling-points or vapour pressure curves are not known in most cases. A suitable way of overcoming this difficulty is to analyse the process using two reference substances, one a fairly volatile substance and the other a substance of lower volatility.

McDowall (4) has used diacetyl* (B.P. 88° C.) and acetoin† (B.P. 150° C.) for experiments with the Vacreator. These substances have boiling-points which make them suitable for reference substances, and in this paper equilibrium values of much the same order as those for diacetyl and acetoin will be used. Neither the vapour pressure curves of the pure substances nor the vapour pressures above solutions in water or cream were available, but estimated values can be predicted along the lines indicated in Appendix 3. From the known or calculated vapour pressure above the solution, the manner in which the reference substance distributes itself between the gas phase and the liquid phase can be calculated.

If the concentration of taint in the gas phase y_e‡ is in equilibrium with the concentration of taint in the liquid phase x_e the value of y_e/x_e is called the equilibrium constant, here given the symbol m. As shown in Appendix 3, diacetyl in water can be expected to have an approximate value of $m=17$. Acetoin in water can be expected to have an approximate value of $m=1{\cdot}4$. Hence these values of $m=17$ and $m=1{\cdot}4$ can be used as limiting values, and although any other suitable pair of values could be used for the purposes of this paper the above values relate to known substances which can be used as reference 'taints'.

Thus characteristic A in Fig. 1 represents equilibrium values for a taint fairly easily removed, and B similarly represents a substance considerably more difficult to strip. Curve C illustrates the case of a non-linear characteristic and shows the change in $m=y_e/x_e$ as the value of x_e changes.

Thus if the performance of a piece of deodorizing equipment is analysed for substances having extreme values of $m=17$ and $m=1{\cdot}4$ for the cream or other liquid processed, the practical performance can be assessed.

THE EFFICIENCY OF CONTACT METHODS

Fig. 2 represents a processing unit for removing a volatile tainting substance from some aqueous liquid feed such as water or cream. The impure feed is fed to the mixer at a rate of L lb./hr. and at a temperature corresponding to the boiling-point in the mixer. Fresh dry saturated steam also at this temperature and pressure is fed to the mixer at a rate of

* $CH_3.CO.CO.CH_3$ (2, 3-butanedione).

† $CH_3.CO.CHOH.CH_3$ (acetyl methyl carbinol).

‡ For nomenclature see p. 365.

G lb./hr. Assuming negligible transfer of heat within the mixer, the rate of flow of steam leaving the system is equal to that entering, and it is shown in Appendix 1 that where the taint transfer has come to equilibrium, the concentration of taint x_0 in lb./lb. in the outgoing liquid can be related to the taint concentration x_1 in lb./lb. in the incoming liquid by the expression

$$\frac{x_0}{x_1}=\frac{1}{1+F},$$

where F is the stripping factor mV/L, m is the ratio of the equilibrium concentrations, V is the lb./hr. of steam leaving the system and equal to G in this case.

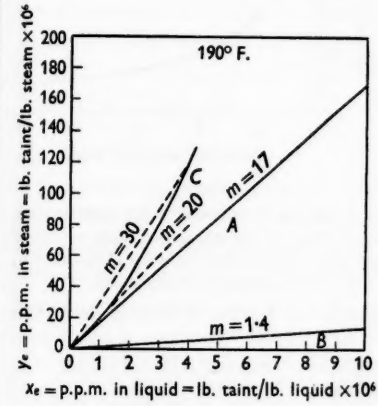
190° F.
m=30
m=20
m=17
m=1·4
A
B
C
y_e = p.p.m. in steam = lb. taint/lb. steam ×10⁶
x_e = p.p.m. in liquid = lb. taint/lb. liquid ×10⁶
0 20 40 60 80 100 120 140 160 180 200
0 1 2 3 4 5 6 7 8 9 10

Fig. 1. Equilibrium distribution of taints between steam and liquid. *A*, more volatile taint; *B*, less volatile taint; *C*, effect of curved characteristic.

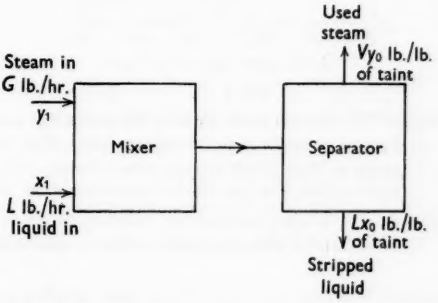

Fig. 2. Diagram of a single stage deodorizing unit.

A plot of this equation is shown in Fig. 3 for two values of m ($m=17$ and $m=1{\cdot}4$) respectively, over a range of steam to liquid ratios.

It is at once obvious from Fig. 3 that for a taint having an equilibrium constant of 17, the use of increasing amounts of steam beyond $V/L=0{\cdot}20$ does not reduce the taint concentration at a rapid rate, and in the deodorization of cream for buttermaking it probably would not be economic to use large quantities of steam to effect a small extra degree of taint removal.

For a tainting substance of considerably lower volatility and represented by an m value of 1·4 the extra increase in taint removal by the use of increasing quantities of steam is very much greater than in the case of a volatile taint. Correlation of the curves in Fig. 3 and the effectiveness of steam in removing taints of any volatility can be represented by a plot of x_0/x_1 against F as shown as the full line in Fig. 4.

Either a low value of m or a low value of steam per lb. of liquid (or the product of the two) will give a small value for the stripping factor F, and operation will be on the initial part of the curve where there is a relatively large change in taint concentration for a small change in the stripping factor.

Since Fig. 4, which is a plot of $x_0/x_1=1/(1+F)$ is based only on a material balance, it can be used for any single contact system where $V=G$ and the value of m for the taint in question is known. Where the system comes to equilibrium m is the equilibrium constant,

but otherwise m represents the ratio of concentration of taint in the outgoing steam to concentration in the outgoing liquid, and could be taken as a pseudo-equilibrium constant for the apparatus.

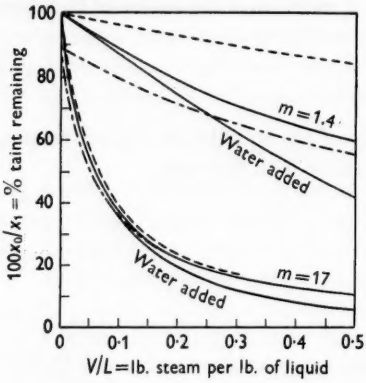

Fig. 3.

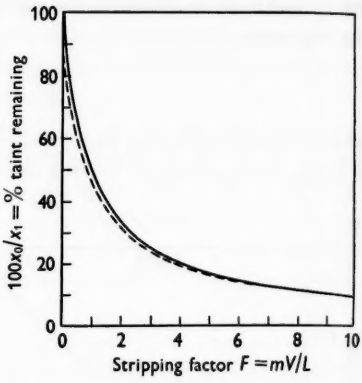

Fig. 4.

Fig. 3. The effect of steam rates on the percentage residual taint. Top group of curves for a less volatile taint. Bottom group for the more volatile taint. Full lines: no dilution and open steam; dotted lines: generated steam or flash steam causing concentration; chain lines: the effect of cold feed causing 15% dilution; water added lines: the effect of replacing water in outgoing liquid for generated or flash steam.

Fig. 4. Correlation of taints of any volatility by means of a stripping factor. Full line: no dilution; dotted line: 15% dilution caused by condensation in cold feed.

THE USE OF A FLASH VESSEL

If an aqueous liquid is allowed to flow into a vessel, held at some pressure below the saturation pressure corresponding to the temperature of the incoming liquid, flash steam will be formed, and under suitable conditions the taint concentration in this flash steam will be in equilibrium with the concentration of taint in the outgoing liquid.

As shown in Appendix 2 the equations representing taint removal conditions in a flash vessel are similar to those for a single contact process but the final taint concentration is greater because the outgoing liquid is concentrated. The particular curves are shown dotted in Fig. 3.

The general equation is

$$\frac{x_0}{x_1} = \frac{1}{(1 - V/L) + F},$$

where $F = mV/L$, L is the feed flow and V the flash steam formed, both in lb./hr.

The method is a particular case of equilibrium distillation, and the pertinent equations and graphical analyses can be used for taint removal calculations.

Fig. 3 shows that there is little reduction in taint concentration by flashing for the lower volatile, but for the more volatile substances the concentration is only slightly greater than where open steam is used.

OPEN STEAM AND GENERATED STEAM

The type of apparatus illustrated by Fig. 2 represents stripping of a taint from a liquid by the use of steam supplied from an external source. Such steam is known as open steam and the stripping action when dealing with an aqueous liquid is in no way different from steam which is boiled from the liquid, as in the case of flash steam.

The amount of flash steam formed could be increased by re-circulation of the liquid, but for milk or cream it seems that this would be unsuitable treatment. By supplying heat from an external source the requisite steam could be boiled off from the aqueous liquid, the same material balance equations applying as for flash steam.

The advantage of open steam is that it does not concentrate the liquid so that the final taint concentration when open steam is used is less than occurs when the equivalent quantity of heat is used to boil off steam from the liquid. Because the final taint in the butter depends on the concentration of taint in the cream from which it was made, the use of open steam is advantageous. An amount of water equal in weight to the water vapour boiled off can be added to the outgoing liquid, and the final taint concentration would then be reduced to a value $(1-V/L)$ of the taint concentrate leaving the boiling apparatus. In this case

$$\frac{(x_0)_d}{x_1}=\frac{1}{1+\dfrac{mV}{L\,(1-V/L)}},$$

and is plotted in Fig. 3.

The value of this procedure is apparent from comparison of the curves. For an equal expenditure of heat the procedure of boiling, and replacing the vapour by added water is seen to give a somewhat lower taint concentration than occurs with open steam usage because concentration has allowed the exit steam to be in contact with liquid having a higher concentration of taint. In normal distillation practice the diluent effect of open steam can be a disadvantage, but in the deodorization of cream the relative diluent or concentration effects must be assessed differently.

Taint stripping of cream by steam is essentially a distillation process and a rigorous method of analysis would be based on enthalpy-concentration differences(2), but because the heat required to vaporize the tainting substance is very small the analytical approach is best made by way of material balances in a manner usually associated with gas absorption theory(9) or liquid extraction(3, 6, 10) theory. The concepts of liquid extraction theory and practice are of particular assistance in the study of steam stripping equipment.

APPLICATION TO THE TANDEM VACREATOR

Fig. 5 shows in diagrammatic form a Tandem Vacreator in which the cream or test liquid is entering the mixer at 190° F., the same temperature as the liquid leaving. The flow of cold water into each of the ejector condensers is usually about 20,000 lb./hr. The temperature rise of the condenser water is usually about 110° F. at a cream flow of (typically) 9000 lb./hr. Thus the steam used for taint removal is about 0·25 lb./lb. of cream, although in practice any figure of 0·1–0·5 lb. of steam per lb. of cream may be used(8), depending on the intensity of treatment desired. In the cooling section the flash steam formed is equal to about $6\frac{1}{2}\%$ of the weight of the cream flowing.

From Fig. 3 the taint removal in each section can be read for constant values of m equal to 17 and 1·4. Thus for the more volatile taint 19% remains in the liquid leaving the mixer separator, and a further 50% reduction takes place in the cooling vessel. The final taint is thus reduced to 9·5% of the input concentration. For the lower volatile taints these figures are 75 and 3%, giving a final remaining concentration of 72·8% of the input concentration.

The difference in taint removal is very striking in this example, and shows very clearly

the difficulty in removing the less volatile taints. In practice the volatilities probably vary over a wide range, the degree of deodorization treatment being set by the desire to minimize the least volatile of the objectionable taints.

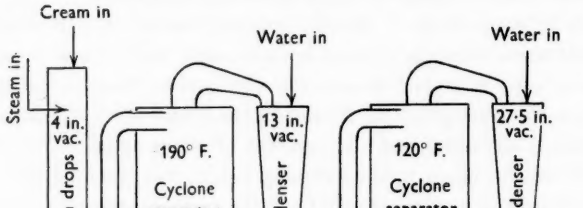

Fig. 5. A Tandem Vacreator consisting of a mixing and separating unit followed by a flash steam cooling vessel.

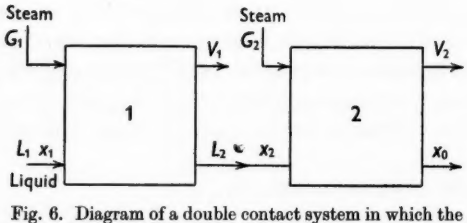

Fig. 6. Diagram of a double contact system in which the total deodorizing steam is divided between two stages.

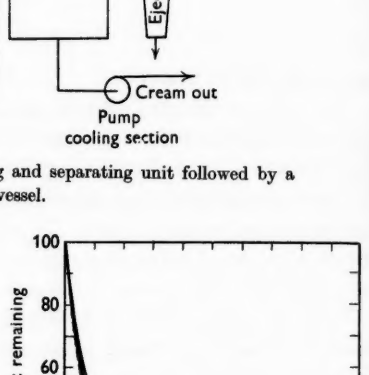

Fig. 7. The effect of the stripping factor on the percentage residual taint. *A*: single contact; *B*: double contact *C*: triple contact; *D*: infinite number of contacts.

DOUBLE CONTACT

Fig. 6 illustrates a double contact system in which fresh steam is supplied to the second, as well as the first mixing (and separating) unit. As for the case of single contact the liquid is assumed to be at the saturation temperature in each case so that neither condensation nor flash steam evolution takes place.

The taint is reduced to a fraction $1/(1+F_1)$ in the first unit and to a further fraction $1/(1+F_2)$ in the second unit. The final fraction for the case where $V_2 = V_1$ and $F_2 = F_1$, is

$$\frac{x_0}{x_1} = \left(\frac{1}{1+mV/2L}\right)^2$$

shown plotted as curve B in Fig. 7. In this equation $G = V$ is the total steam supplied to both units.

For comparison the curve A for single contact operation is plotted, and shows the superiority of the double contact method, either as a means of accomplishing a required degree of taint removal with less steam or further reducing the taint with the use of the same quantity of steam.* The divergence between the curves becomes greater at high values of the stripping factor so that in practice the maximum steam saving is made when the critical taint is reasonably volatile. Thus if the final (volatile) taint is to be 10% of the initial, the double contact system will use about half the steam required for the single contact arrangement.

APPLICATION TO THE TRIPLE VACREATOR

Many of the butter factories have found it necessary to add a further deodorizing stage to the Tandem Vacreator. The complete unit becomes a double contact process followed by a flash cooling vessel and the name Triple or Three Leg Vacreator has been given to the system.

A typical steam consumption is 0·20 lb./lb. of cream (for deodorizing purposes) in each of the two stages. For a taint having an equilibrium constant m of 17 the overall performance can be evaluated from Fig. 3 as $22\% \times 22\% \times 50\%$ or 2·42% of the taint concentration entering. For a taint having an equilibrium constant of 1·4 and using the same unit steam consumption the final taint as evaluated from Fig. 3 is $78{\cdot}5\% \times 78{\cdot}5\% \times 97\%$ or 60% of the original.

MULTIPLE CONTACT SYSTEMS

The double contact method in which fresh steam is supplied to two mixers can be extended to three or more stages. For the cases where the feed is at saturation temperature the general equation is

$$\frac{x_0}{x_1} = \left(\frac{1}{1+F/N}\right)^N$$

for constant m and equal quantities of steam in each of the N vessels. This equation is plotted as curve C in Fig. 7 for three vessels. The gain over double contact is not considerable and is rapidly asymptotic to $x_0/x_1 = 1/e^F$ shown as the dotted curve, and indicates clearly the limitations of the multi-contact system. When N is very large, i.e. when there are many stages the value of $\left(\frac{1}{1+F/N}\right)^N$ approaches $1/e^F$, $(e = 2{\cdot}718)$ hence a plot of $x_0/x_1 = 1/e^F$ represents the theoretical minimum taint residue when the total steam used is evenly divided amongst an infinite number of stages. It seems likely that the gain due to the use of more than about three stages would scarcely offset the cost and complication of the extra equipment. More particularly is this true where reasonably volatile taints have to be stripped.

It can be shown that for maximum taint removal the total deodorizing steam should be evenly divided among the N stages.

LIQUID TEMPERATURES BELOW SATURATION TEMPERATURE

If the cream or other liquid fed to a mixing vessel is at a temperature below the saturation temperature existing in the vessel, some of the steam will condense and heat the liquid

* McDowall (private communication) has demonstrated this experimentally with a Vacreator.

to saturation temperature. The final taint concentration for this case is derived in Appendix 2 and for a single contact is

$$\frac{x_0}{x_1}=\frac{1}{\left(1+\frac{S}{L}\right)+\frac{mV}{L}},$$

where S/L is the lb. of dilution per lb. of liquid fed and V/L is the lb. of steam issuing from the vessel per lb. of liquid feed entering. Heating the cream from room temperature to the exit temperature in a Vacreator unit adds about 12·5% dilution to the cream, and the chain curve in Fig. 4 and the chain curves in Fig. 3 are plots of the above equation where $S/L=0{\cdot}125$.

Dilution has little effect on the final concentration of a substance represented by an m of 17 but there is quite an appreciable lowering of the x_0/x_1 ratio for a substance having an m of 1·4, especially where the steam-to-liquid ratio is small.

GRAPHICAL ANALYSIS

Single and multiple contact

The equations so far derived have been calculated on the assumption that the equilibrium relationships between the concentrations of taint in the steam and the concentration of taint in the liquid is a linear one. This is approximately true in some cases but a more general method of analysis must be able to take into account an equilibrium relationship

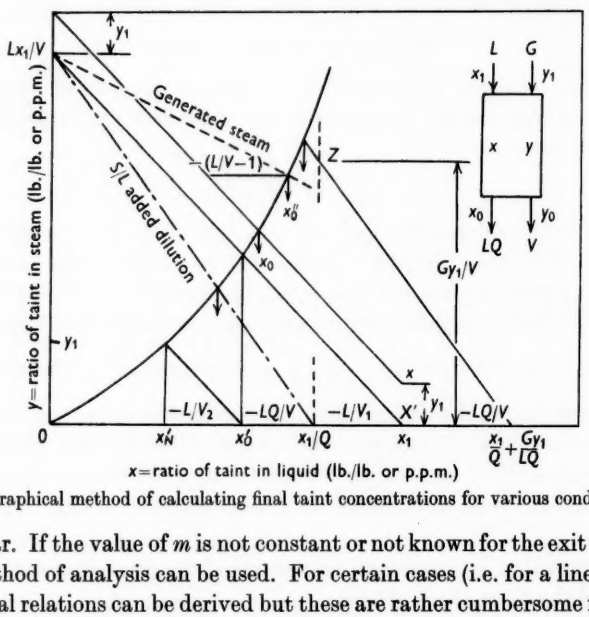

Fig. 8. Graphical method of calculating final taint concentrations for various conditions.

that is not linear. If the value of m is not constant or not known for the exit concentrations a graphical method of analysis can be used. For certain cases (i.e. for a linear variation of m) mathematical relations can be derived but these are rather cumbersome for normal use.

The inset diagram of Fig. 8 represents a parallel flow or mixing type of stripping unit. Irrespective of the shape of the equilibrium curve, at any point

lb. of taint taken up by the steam = lb. of taint removed from liquid,

or $$V\,(y-y_1)=L\,(x_1-x), \quad \text{thus} \quad y=\left(\frac{Lx_1}{V}+y_1\right)-\frac{Lx}{V}$$

so that the operating line y versus x is a straight line from the entry conditions

$$X\ (x=x_1 \text{ and } y=y_1)$$

(when the feed is at saturation temperature) having a negative slope equal to the ratio lb. of liquid per lb. of steam. The place of intersection with the equilibrium curve locates the end-point of the separation process. Where there is no taint in the incoming steam the starting-point of the operation line is x' $(x=x_1,\ y=0)$ giving a final taint of x'_0.

For multiple contact systems the analysis can be repeated. Thus using x'_0 as the new origin and by drawing a line of the appropriate slope the concentration at the end of the second stage is given as x_n.

The operating line for generated steam taint removal has a slope of $-(L/V-1)$ (see Appendix 2) and is shown dotted to give a final value of x''_0, which is greater than x'_0.

Where dilution of the liquid occurs the appropriate operating line equation from Appendix 2 can be plotted. Thus for the common case where dilution occurs, but there is no taint in the incoming steam, the operating line is

$$y=\frac{Lx_1}{V}-\frac{LQx}{V} \quad \text{from equation (A 2·3),}$$

where Q is the ratio of outgoing liquid to inflow liquid. The construction is shown as a chain line in Fig. 8 where it is obviously a particular case in which the original concentration is reduced to x_1/Q and the slope of the line increased to $-LQ/V$.

Should the incoming steam contain some of the tainting material then the operating line is

$$y=\left(\frac{Lx_1}{V}+\frac{Gy_1}{V}\right)-\frac{LQx}{V} \quad \text{from equation (A 2·1).}$$

A plot of this equation is shown as the top line, passing through the point Z with a slope $-LQ/V$.

USE OF THE ANALYSIS FOR EQUILIBRIUM VALUES

The equation representing the operating lines, or the graphical analysis, can be used to plot the relationship between the exit concentration in the steam, y_0, against the concentration in the liquid, x_0, from experimental data, when both x_1 and x_0 are known. If the process has come to equilibrium then the y_0-x_0 curve represents the equilibrium curve for the solute.

Some data from experiments (reported in Part II of these papers) were used in the appropriate equations for the computation of equilibrium points for diacetyl in water. Similarly, the equilibrium points for acetoin in water were calculated. There was some scattering of the points (especially in the case of acetoin, and probably caused by variable dilution effects due to changing steam wetness conditions), but an averaging curve representing the approximate equilibrium characteristic could be drawn.

Table 1 lists the points representing the average curves.

The results given in Table 1, derived in the manner indicated, are approximate only. Furthermore, the diacetyl used in the experiments may not have been pure so that there may be differences between the values given in Table 1 and values determined in a laboratory equilibrium still. For the direct comparison of one type of plant with another, or for measuring the effect of plant changes on steam demand, purity of the reference substance is not important provided there is no change in the purity of the reference substance during the course of the experiments.

By means of the graphical analysis presented and the equilibrium characteristics

Table 1. *Approximate equilibrium characteristics for diacetyl and acetoin water*

(Range 190–210° F.)

Concentration in water (p.p.m.)	Equilibrium concentration in steam above the water (p.p.m.)	
	Diacetyl	Acetoin
0	0	0
1	18	0·8*
2	45	1·8*
3	65	2·6
4	80	4·0
5	97	5·5
6	—	7·4
7	—	9·5
8	—	11·7
9	—	14·0
10	—	16·0

* By extrapolation only.

shown in Table 1 the degree of removal of diacetyl from water was computed for a range of steam flows and for a dilution of 15% caused by condensation of steam into the cold water entering; the results are plotted in Fig. 9. For comparison are shown some experimental points as obtained by McDowall. In a similar manner the computed curves for acetoin removal and experimental points of McDowall are compared in Fig. 9.

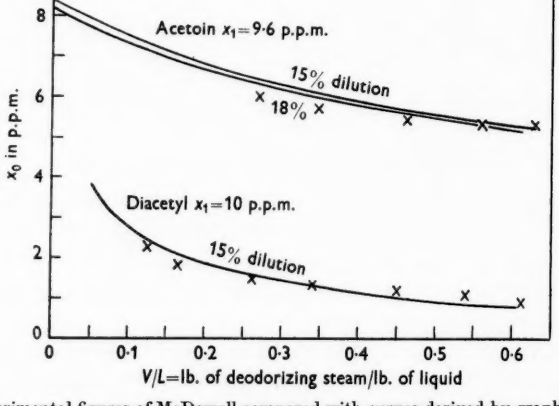

Fig. 9. Experimental figures of McDowall compared with curves derived by graphical analysis for solo section of small Vacreator.

Agreement of the diacetyl points is better than for the acetoin points and this would be expected since dilution has less effect on the final concentration for diacetyl. Experimental determinations of diacetyl concentrations are more accurate than similar determinations for acetoin and this may be a contributing cause of better agreement of the diacetyl computed and experimental points.

SUMMARY

The operation of equipment for reducing the amounts of volatile taints in liquids by the use of open or generated steam is explained and analysed. From consideration of material balances for each system and the equilibrium characteristics of the volatile substances,

formulae and graphical methods are derived and used to show the degree of taint removal which can be attained by the steam. The methods and charts are used to show the performance of Tandem and Triple Vacreators. The methods presented in the paper can be used for any volatile solute but particular reference is made to two substances having equilibrium constants of 17 and 1·4 because these represent a range of volatilities of particular interest and are approximate values for diacetyl and acetoin which can be used as reference substances.

The effectiveness of any system is shown to depend on the value of a stripping factor which is equal to the product of the equilibrium constant and the pounds of deodorizing steam per lb. of liquid (i.e. $F = mV/L$). The efficiency of methods where the liquid is contacted several times with fresh steam is shown to be much greater than for single contact. The gain in efficiency for each stage becomes less pronounced with increasing contacts and the maximum efficiency is shown to be limited even for an infinite number of contacts.

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NOMENCLATURE

e = 2·718.
F = stripping factor mV/L. Dimensionless, F_1 for first vessel and F_2 for second vessel.
G = inflow of steam to any system (lb./hr.).
L = inflow of liquid to any system (lb./hr.).
m = equilibrium constant = y_e/x_e at any point.
N = number of contacts in a multi-contact system.
Q = dilution factor = $1 \pm S/L$, dimensionless.
S = weight of steam condensed and adding to the liquid flow (lb./hr.).
$-S$ = weight of flash steam formed and subtracted from liquid flow (lb./hr.).
V = outflow of steam in any system (lb./hr.).
x = concentration of taint in liquid at any point (lb./lb.).
x_1 = concentration of taint in incoming liquid (lb./lb.).
x_2 = concentration of taint in liquid leaving first vessel and entering second vessel (lb./lb.).
x_0 = concentration of taint in liquid leaving a system of one or two contacts (lb./lb.).
$(x_0)_d$ = concentration of taint in liquid leaving, which has been diluted with water after steam stripping (lb./lb.).
x_e = concentration of taint in liquid which is in equilibrium with y_e (lb./lb.).

y = concentration of taint in steam at any point in a system (lb./lb.).
y_1 = concentration of taint in steam entering a system (lb./lb.).
y_0 = concentration of taint in steam leaving a system (lb./lb.).
y_e = concentration of taint in steam which is in equilibrium with x_e for any value of x_e (lb./lb.).

APPENDIX I

Taint balance for fresh steam and constant flows

Fig. A 1 represents a contacting vessel in which the liquid has a constant flow of L lb./hr. with inlet and exit taint concentrations x_1 and x_0 expressed in lb./lb. of taint-free liquid. The steam flow is constant at V lb./hr. and the taint concentration increases from zero to y_0 lb./lb. of taint-free vapour. There is no dilution or flash steam, so that the weight of steam entering is equal to the weight of steam leaving.

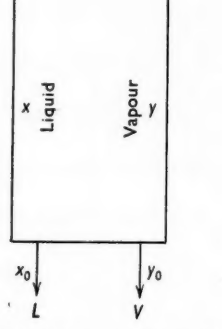

Fig. A 1. Material balance conditions for a single stage in which no dilution or concentration occurs and incoming steam contains no taint.

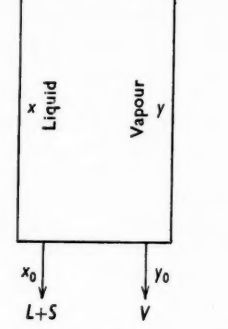

Fig. A 2. Material balance conditions for general case.

Since the weight of taint taken up in unit time by the steam must equal the weight of taint lost by the liquid in the same time, $Vy = Lx_1 - Lx$ at any point. Hence the operating line equation is

$$y = \frac{Lx_1}{V} - \frac{Lx}{V}. \qquad \text{(A 1·1)}$$

If the relation between y_0 and x_0 is known equation (A 1) can be solved. Thus if equilibrium is reached $y = mx$ at the exit point and $mVx_0 = Lx_1 - Lx_0$. Hence

$$\frac{x_0}{x_1} = \frac{1}{1+F} \quad \text{where} \quad F = \frac{mV}{L}.$$

APPENDIX II

Taint balance for general case

In this case the inflow of steam is G lb./hr., of which S lb./hr. condense so that the liquid flow L is increased to $L+S$ lb./hr.

A taint balance gives $Lx_1+Gy_1=(L+S)\,x+Vy$ and the operating line equation is

$$y=\left(\frac{Lx_1}{V}+\frac{Gy_1}{V}\right)-\frac{LQx}{V}, \tag{A 2·1}$$

where $$Q=\frac{L+S}{L}=\left(1+\frac{S}{L}\right).$$

Putting $y=mx$ as the exit relationship $mVx=Lx_1+Gy_1-LQx$

$$\frac{x_0}{x_1}=\frac{1+\left(\frac{G}{L}\frac{y_1}{x_1}\right)}{Q+F}, \tag{A 2·2}$$

where $$F=mV/L.$$

Particular cases can be derived from the general case as follows:

Condensation but no initial taint in steam ($y_1=0$)

Operating line $$y=\frac{Lx_1}{V}-\frac{LQx}{V}. \tag{A 2·3}$$

Final concentration ratio $$\frac{x_0}{x_1}=\frac{1}{Q+F}. \tag{A 2·4}$$

Initial taint in steam but no condensation ($S=0$)

Operating line $$y=\left(\frac{Lx_1}{V}+y_1\right)-\frac{Lx}{V}. \tag{A 2·5}$$

Final concentration ratio $$\frac{x_0}{x_1}=\frac{1+\left(\frac{V}{L}\frac{y_1}{x_1}\right)}{1+F}. \tag{A 2·6}$$

No initial taint in steam and no condensation ($y_1=0$, $S=0$)

Operating line $$y=\frac{Lx_1}{V}-\frac{Lx}{V}. \tag{A 2·7}$$

Final concentration ratio $$\frac{x_0}{x_1}=\frac{1}{1+F}. \tag{A 2·8}$$

Flash steam and generated steam

Where some of the feed forms flash steam the equations remain unchanged, but with $S=-S$. The particular case where no steam is supplied, i.e. $G=0$ and $S=V$ gives:

Operating line $$y=\frac{Lx_1}{V}-\frac{L\left(1-\frac{S}{L}\right)x}{V}$$

$$=\frac{Lx_1}{V}-\left(\frac{L}{V}-1\right)x. \tag{A 2·9}$$

Final concentration ratio $$\frac{x_0}{x_1}=\frac{1}{Q+F}=\frac{1}{\left(1-\frac{V}{L}\right)+F}. \tag{A 2·10}$$

Where external heat is supplied and steam is generated from the water in the liquid the equations are the same as those given for flash steam.

Liquid added to replace generated steam

If an amount of water equal to V lb./hr. is added to the outgoing liquid to replace the V lb./hr. removed as vapour, then the liquid flow resumes its rate of L lb./hr. and the new exit taint concentration becomes $(L-V)\,x_0/L$ and

$$\frac{(x_0)_d}{x_1}=\frac{\text{Final taint concentration}}{\text{Original taint concentration}}=\frac{(1-V/L)\,x_0}{x_1}=\frac{(1-V/L)}{(1-V/L+F}=\frac{1}{1+\dfrac{mV}{(1-V/L)\,L}}.$$

APPENDIX III

Equilibrium constants

The equilibrium concentrations of a small quantity of a reference substance distributed between a gaseous phase and a liquid phase can be calculated if the vapour pressure of the substance in the gaseous phase is known. Because the amounts of the substances (in lb.-mol.) are proportional to their partial vapour pressures,

$$y_e=\frac{M_r}{Mg}\frac{P_v}{P}, \qquad \text{(A 3·1)}$$

where

y_e = lb. of taint or reference substance per lb. of taint-free gas or vapour;
M_r = molecular weight of reference substance;
Mg = molecular weight of gas or vapour;
P_v = partial vapour pressure of reference substance above the solution;
P = total pressure of the gas mixture.

Thus if the vapour pressures P_v are known for values of x_e, the equilibrium concentration in the liquid phase, the corresponding values of y_e can be calculated and the curve of y_e versus x_e can be drawn.

If values of P_v corresponding to values of x_e are not available from experimentally determined figures it is sometimes possible to estimate the P_v-x_e curve. Thus for a dilute solution

$$P_v=P_a x_e \frac{M_L}{M_r} f, \qquad \text{(A 3·2)}$$

where

P_a = vapour pressure of pure reference substance at same temperature as solution;
x_e = the equilibrium concentration of reference substance in the liquid phase, lb./lb. pure liquid;
M_L = molecular weight of liquid;
f = activity coefficient.

Substituting the value for P_v from equation (A 3·2) in equation (A 3·1) we get

$$y_e=f\frac{M_L}{Mg}\frac{P_a}{P}x_e, \qquad \text{(A 3·3)}$$

or $y_e=mx_e$, where $m=f\dfrac{M_L}{Mg}\dfrac{P_a}{P}$.

In order to use equation A 3·3 to predict the equilibrium curve, values of the activity coefficient must be known or estimated for the particular conditions of temperature and concentration. Similarly, the vapour pressure of the pure reference substance must be known (or predicted by some means such as the Duhring plot) for the required temperature.

When the analysis presented in the paper was commenced no equilibrium data was available for diacetyl or acetoin, and values of $m = 17$ for diacetyl and $m = 1{\cdot}4$ for acetoin were predicted in the manner indicated above. Since then approximate experimental data is available (see Part II) but the above values have been retained.

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563. THE STEAM STRIPPING OF TAINTS FROM LIQUIDS

II. COUNTERFLOW STRIPPING WITH PARTICULAR REFERENCE TO POSSIBLE USE OF VACREATOR EQUIPMENT

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(With 9 Figures)

INTRODUCTION

The amount of taint removed per lb. of steam in any process depends on the concentration of taint in that steam. The concentration of taint in the steam, in turn, depends largely on the concentration of taint in the liquid which is in contact with the outgoing steam. Thus the degree of taint removal which can be obtained by any process can be improved by arranging for all the steam (or as much as possible) which leaves the system to be in contact with liquid of a high taint concentration.

The multi-contact method described in Part I (7), in which the flow of outgoing steam for each stage was in contact with liquid of increasing taint concentration, carried out the above principle to some degree, but the final effect was not as great as could be obtained by counterflow methods.

In a counterflow column the steam enters the system (usually at the bottom) at the place where the stripped liquid leaves. The outgoing steam leaves the system at the top where the liquid enters, and is thus in contact with a liquid having the highest taint concentration. The lower inset diagram of Fig. 6 shows such an arrangement. If the rate of transfer is great, or the height of the column is great, the concentration of taint in the steam is very nearly in equilibrium with the concentration of taint in the liquid.

Counterflow movement of the cream and the steam is possible in several ways, but restrictions of foaming or the need for easy cleaning limit the design of counterflow columns, and the bubble cap and packed columns (1, 4) of the chemical industry have not so far been used in cream processing. The simple arrangement of a downward spray of cream drops and an upward flow of steam is possible and has been used (3). It will be shown in a later paper that under these conditions the rate of transfer of taint is expected to be low unless the cream drops are continually broken and reformed. The cream can also be made to pass down the sides of the column in a thin film but here again it can be shown that unless special precautions are taken the gain due to the greater driving force in a single counterflow stage as compared with the driving force (causing the movement of taint from the liquid into the steam) in a parallel flow or mixing stage, may be partly or wholly offset by the reduced rate of taint movement per unit difference of driving force.

Since equilibrium is never attained in a practical counterflow column, the degree of taint removal is associated with the study of rates of taint transfer which will be presented in a later paper of this series. An alternative to continuous counterflow is multiple contact counterflow (5, 6) whereby two or more mixing (or parallel flow) stages reaching equilibrium within each stage, are arranged in counterflow in a manner to be described and indicated in Fig. 1.

MULTIPLE CONTACT COUNTERFLOW

Fig. 1 is a diagram of a double contact system in which all the steam which enters the system is supplied to no. 2 stage. Although the concentration y_0† of taint in the steam leaving this vessel is in equilibrium with the concentration of taint x_0 in the liquid leaving, y_0 is less than y_1† the taint concentration which WOULD be in equilibrium with x_1. Thus instead of supplying fresh steam to stage 1 the exhaust steam from stage 2 can be used again so that further taint removal can be effected before the steam is finally led to waste.

The system can be extended to three (or more stages) as shown dotted and can be seen to be equivalent to a countercurrent system of three theoretical plates.

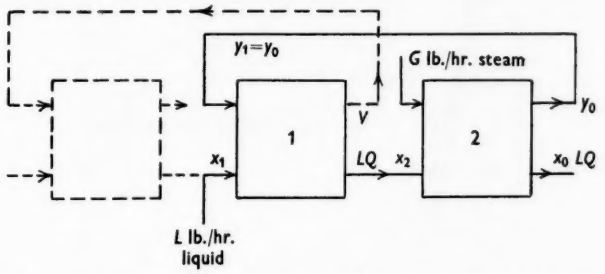

Fig. 1. Diagram of a double contact counterflow system. Additional stage for triple contact shown dotted.

(If the taint in the liquid stream leaving any piece of deodorizing apparatus is such that the concentration would be in equilibrium with the concentration of taint in the exit steam (if the exit streams were placed in contact) then such a unit is called a theoretical plate. From the point of view of taint removal a theoretical plate gives exactly the same results as a mixing unit or a parallel flow unit in which equilibrium is reached. Complete equilibrium is impossible in practical parallel flow equipment but where the vapour and liquid are well mixed a state very close to equilibrium can be attained.)

As shown in the Appendix the equations for systems supplied with feed at the saturation temperature are:

two stages $$\frac{x_0}{x_1}=\frac{F-1}{F^3-1},$$

three stages $$\frac{x_0}{x_1}=\frac{F-1}{F^4-1},$$

N stages $$\frac{x_0}{x_1}=\frac{F-1}{F^{N+1}-1},$$

where $F=mV/L$.

The equations for two, three and four stages are plotted in Fig. 2 as curves *E–G*. Fig. 2 shows also the multi-contact curves which were derived and discussed in Part I. Their inclusion here makes possible a ready comparison between multi-contact and counterflow systems, especially for design use. The large improvement over simple multiple contact is at once apparent, and comparison with the curve for an infinite number of multiple contacts shows that even a three-stage counterflow system is con-

† For nomenclature see p. 381.

siderably better. As would be expected, Fig. 2 shows very clearly that for taints of low volatility, say $m = 1{\cdot}4$, giving low values for the stripping factor, a high degree of taint removal is not possible unless large amounts of steam are used. The steam saving possible by the use of counterflow is very much less for taints of low volatility than for taints more easily removed.

Curves *E*–*G* also show the results of taint stripping in a countercurrent tower of two, three or four theoretical plates.

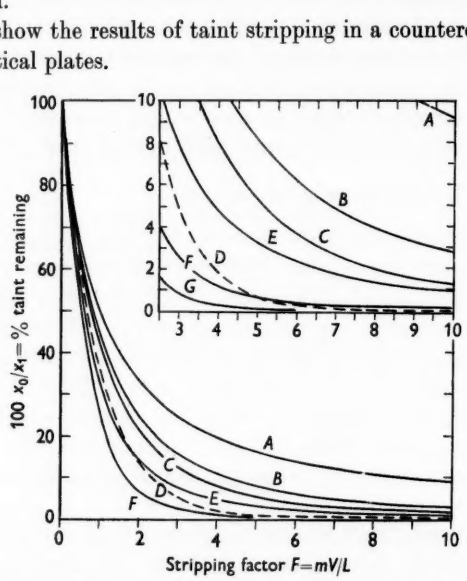

Fig. 2. The effect of the stripping factor on the percentage residual taint. *A*, single contact; *B*, double contact; *C*, triple contact; *D*, infinite number of contacts; *E*, double contact countercurrent; *F*, triple contact countercurrent; *G*, quadruple contact countercurrent.

POSSIBLE APPLICATION OF MULTI-CONTACT COUNTERFLOW IN PRACTICE

Most of the steam used in buttermaking practice in New Zealand is used in Vacreators (7) for the removal of taint. The low efficiency of the Vacreator as a means of removing taint suggests that considerable economies could be made by the use of some form of countercurrent flow in the deodorizing operation. A design based on a tower suitable for the countercurrent steam stripping of taints from cream would have many advantages especially if it were equal to three or four theoretical plates. On the other hand, there are a considerable number of Vacreators in use and it would be desirable to use these existing units with multi-contact counterflow connexions.

In Fig. 3 showing a Triple Vacreator, if the goose neck and ejector condenser marked *Y* and *X* are removed and replaced by a large diameter pipe (shown dotted) for the re-use of the steam, the system is converted to a double contact counterflow system in which the condenser on no. 1 mixer holds the desired vacuum in both units and condenses all the steam. The first mixer will be at a slightly higher vacuum (and lower temperature) than the second mixer. In the event of one condenser being too small in capacity, the surplus condenser *X* could be connected in parallel with the operating one. The diameter of the steam connexion between the two units should be large enough to convey the low pressure steam without a large loss in pressure. The steam supply to the no. 1 unit is, of course, turned off.

For those butter factories which have Tandem Vacreators installed there are several courses open. Another mixing and separating unit can be obtained and added to the existing units giving an arrangement as shown in Fig. 3. In all probability the cyclonic separator used with standard Vacreator equipment would not be necessary for no. 2 mixer since any entrainment in the steam coming from no. 2 mixer enters the system again and is added to the incoming cream. One would expect a reasonable limit to the amount of entrainment allowable otherwise small portions of the cream would circulate and become over treated.

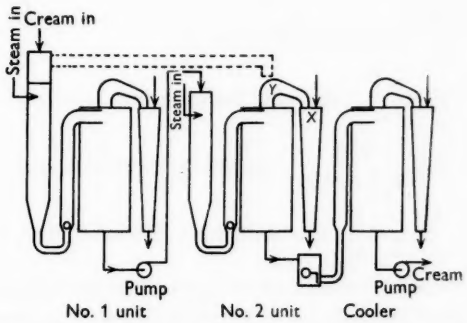

Fig. 3. Triple Vacreator; dotted connexion shows method for countercurrent operation.

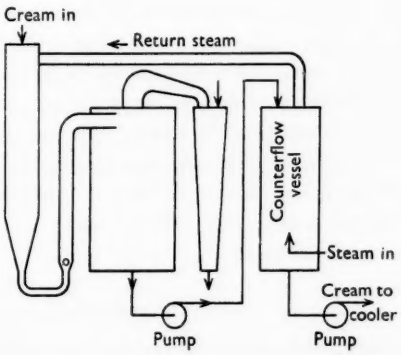

Fig. 4. The addition of a continuous countercurrent section of Z theoretical plates to Vacreator. Complete system equivalent to $Z+1$ plates counterflow.

One other possible arrangement is the use of a counterflow vessel in place of no. 2 mixer. Fig. 4 shows the arrangement with the cooling section omitted.

The system is equivalent to $Z+1$ theoretical plates arranged for counter current flow, where Z is the number of theoretical plates in the counter current column. There appear several advantages in a scheme of this nature, even for a plant of new design because the mixer unit acts as a de-gasser and a foam and entrainment collector as well as adding a further plate to the system.

Experimental results obtained from a converted small size Tandem Vacreator will be given in a later section of this paper for the case where $Z=1$.

EFFECT OF A LOW-FEED TEMPERATURE

The double contact counterflow arrangement shown in Fig. 1 is more efficient (i.e. gives a lower value of x_0/x_1 for the same value of total steam used) when cold liquid enters than when the liquid is separately heated by steam. The reason for this is that the liquid entering the second vessel from the first is hot so that none of the steam G is condensed until it reaches the first vessel. Thus the steam used to heat the liquid is first used for stripping taint from the liquid in the second vessel. The equation for double contact counterflow is derived in the Appendix as

$$\frac{x_0}{x_1}=\frac{1}{(1+S/L)+F_1(1+F_2)},$$

where

$F_1=\dfrac{mV}{L}$ stripping factor in first vessel,

$F_2=\dfrac{m_2G}{L(1+S/L)}$ stripping factor in second vessel,

$\dfrac{S}{L}=\dfrac{G}{L}-\dfrac{V}{L}$ fraction added dilution in first vessel.

The steam coming from the second vessel carries a concentration y_0 of taint, and the inflow of taint Gy_0 lb./hr. is added to the taint entering with the cream, Lx_1 or LQ (x_1/Q) to give rise to an increase in the taint levels in the first vessel, and the steam leaving the system contains a greater concentration of taint than otherwise would be possible. Thus in the first vessel dilution caused by condensation tends to reduce the concentration of taint in the liquid and in turn the concentration of taint in the steam; the taint content in the steam which condenses, raises the concentration of taint in the outgoing steam so that the system incorporates reflux (2) which is always present, but except for low rates of steam flow the reflux effect is masked by the diluent effect of the condensing steam. The reflux effect depends on (*a*) the ratio of the steam entering the first vessel to the steam leaving the vessel, and (*b*) the concentration of taint in the incoming steam. Thus when stripping a reasonably volatile solute from a liquid using small amounts of steam the effect of reflux would be very noticeable.

TOTAL STEAM CONSUMPTION

In addition to the steam required to deodorize cream, a quantity is also required to heat the cream to the saturation temperature corresponding to the pressure in the vessel. In some processing systems all the necessary heating is done in a steam jacketed vessel (commonly known as a flash pasteurizer), in others the cream is partially heated, usually by a plate heat exchanger using waste heat from the deodorizing process; and in other systems the cream is supplied to the deodorizer without having been preheated in any way.

The total steam, including heating steam for a double contact counterflow system is shown in Fig. 5. The curves are based on the approximate equilibrium curve for diacetyl in water and computed by the graphical method. The initial taint concentration is assumed as 10 p.p.m.

Curve *A* represents the relation between the lb. of steam used (per lb. of water) and the residual diacetyl when all the necessary heating is achieved by waste heat. Curve *B* is for

the case where all the preheating in a heat exchanger is done by steam using 0·15 lb./lb. of water. Curve *C* represents the case where the water is fed cold so that 15% dilution takes place in the first vessel. Curve *D* is for a system in which some of the preheat is accomplished by waste heat (heating say to 140° F.) and the remainder by steam condensing in the first vessel giving rise to a 7½% dilution. Comparison of the curves shows the gain caused by the use of the heating steam as part of the deodorizing steam in the second vessel, but in spite of this gain it is desirable to heat with waste heat to as high a temperature as possible.

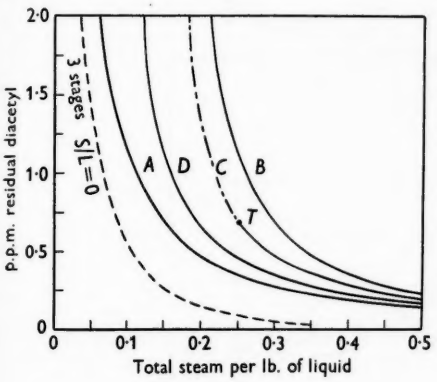

Fig. 5.

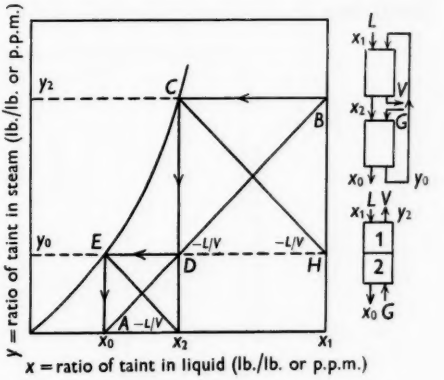

Fig. 6.

Fig. 5. The effect on steam consumption of various methods of heating with double contact counterflow stripping. Diacetyl used as reference substance in water. *A*, all heating done by waste heat; *B*, all heating by external heat exchanger; *C*, cold feed, heating done in first stage by steam coming from second stage; *D*, as for case *C*, but feed partly heated by waste heat.

Fig. 6. Graphical construction for multiple contact counterflow or continuous counterflow where no dilution or concentration of liquid occurs. Illustrated by double contact system.

GRAPHICAL ANALYSIS

Fig. 6 shows for the case of no dilution a graphical analysis of the double contact counterflow system of Fig. 1. The procedure follows that given in Part I.

The position of x_2 must be found by trial. A line drawn from the assumed x_2 at a slope of $-L/V$ gives the final taint concentration x_0 and the concentration of taint y_0 in the steam supplied to the first stage. A line of slope $-L/V$ from the x_1, y_0 point H is drawn and intersects the equilibrium curve at x_2, y_2, if x_2 has been correctly chosen initially. Two or three trials are generally sufficient to locate x_2.

Inspection of the diagram shows that the line AB is the load line for a counterflow tower and the heavy lines are the constructions for a two plate McCabe-Thiele analysis. Hence an alternative construction is to draw the load line AB (to a slope L/V) by trial, draw BC to meet the equilibrium curve at C and drop a vertical line CD to meet the load line at D. Similarly, DE and EA are drawn and if the point A does not coincide with the x_0y_1 point a new trial load line must be made and the concentration repeated. A similar construction is used for a stripping system equivalent to three or more theoretical plates or mixing units arranged for counterflow.

Fig. 7 shows the construction for a case where dilution occurs in the first vessel, and as for the previous case a value of x_2 has to be arbitrarily chosen and a line is drawn at a slope $-LQ/G$ to cut the equilibrium curve at A giving a value for the final taint concentration x_0 and a value $y_0=y_1$ for the taint concentration in the steam supplied to the first unit. A horizontal line AB is drawn to meet a vertical from x_1/Q at the point B and the vertical is extended to point C so that C is $(G/V)\,y_1$ above the x axis. From point C a straight line at a slope $-LQ/V$ is drawn to cut the equilibrium curve at D. If x_2 has been correctly chosen then a vertical from D will meet the $x=x_2$, $y=0$ point. A few trials are usually sufficient to locate the correct value of x_2. If the dilution in the first vessel is S lb. of liquid then $V=G-S$ or $V/L=G/L-S/L$.

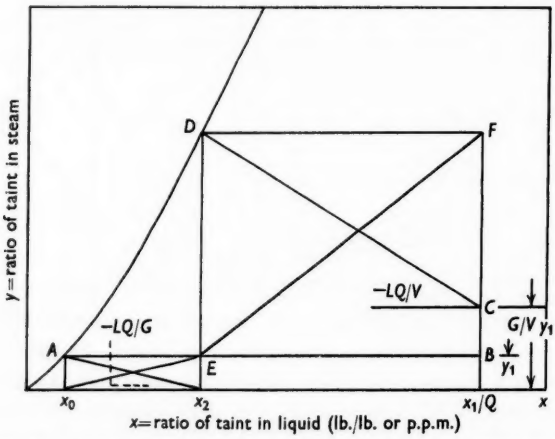

Fig. 7. Graphical construction for multiple contact counterflow where dilution of liquid steam occurs in first stage. Illustrated for double contact system.

When G/L is small the value of G/V is large and hence the value of Gy_1/V can be located at a point above the equilibrium curve and the value of x_2 is larger than x_1/Q. Thus the taint concentration is increased (as would be indicated by a state point above the equilibrium curve) and can exceed the initial value of x_1. The value of x_2 when no steam leaves the system (i.e. when $V=0$) is derived in the Appendix.

EXPERIMENTAL RESULTS

Vacreators are used for all the steam stripping operations in commercial use in New Zealand's butter factories,† and the desirability of using existing Vacreator equipment for counterflow contact operation is evident.

As an initial step a small Tandem Vacreator was converted to double contact counterflow operation as illustrated in Fig. 8 as Type I connexion.

As shown the condenser for the second vessel was disconnected and a pipe connecting the second vessel and the pasteurizing section was placed in position. A centrifugal pump was added, to pump the liquid from the intermediate float tank to the second stage. Fresh steam entered the system at the pump discharge and was mixed with the liquid in a suitable double bent piece of piping.

† Since this paper was written a counter current system is now in commercial operation (3).

The system operated smoothly and no trouble was experienced in the management of the plant over a wide range of temperature and steam flow conditions. Water containing a volatile reference substance was used instead of cream for a series of experiments. As a reference substance either diacetyl or acetoin was used (see Part I). Runs 1 and 2 of Table 1 show the results from the diacetyl tests and runs 2 and 4 of Table 2 give results for acetoin tests. Run 3 (in Table 1) was made with a different method of connecting the Vacreator to that shown in Fig. 8.

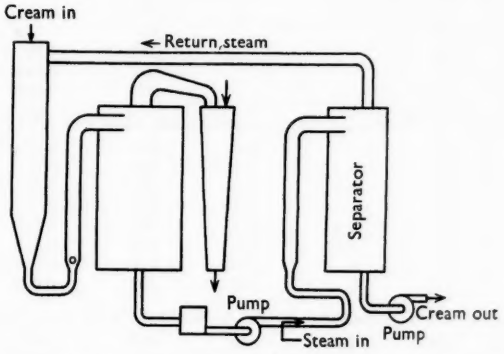

Fig. 8. Tandem Vacreator arranged as double contact counterflow system for experimental purposes.

This latter arrangement was designated as Type II and was effected by allowing the steam to enter the system through the normal steam entry in the pasteurizing chamber, the steam leaving the system by way of the condenser connected to the vessel normally used as the cooling section. The partly used steam was led from the pasteurizer separator to an inlet at the intermediate float tank. The liquid entered the system at the intermediate float tank and was conveyed to the pasteurizing section by a line connected to the normal discharge pump. A pump connected to the pasteurizer separator pumped the liquid from the plant. Thus steam flow was in the normal direction but the cream flow between the vessels was reversed; in Type I the liquid flow was in the normal direction but the steam flow was reversed.

The chemical method used for the determination of the diacetyl (see reference 4, Part I) content of water did not allow concentrations of less than about 0·3 p.p.m. to be determined with any accuracy, and the values marked with an asterisk in Table 1 cannot be taken as correct values.

In addition to the experiments reported here, experimental determination was made of the length of pipe required to cause substantial equilibrium for diacetyl being stripped from water by steam. The mixing tube contained a number of sampling cocks spaced at intervals and the results showed that with 1000 lb./hr. of water flowing through a 1 in. tube near equilibrium was established even for a pipe length as short as 2 ft. The length of pipe used as a contact stage in the counterflow experiments was about 10 ft., before entering the uptake pipe, and included two sharp bends, so that adequate mixing and equilibrium was assured.

Calculations of the equilibrium curves from the experimental results of Tables 1 and 2 gave the figures already reported in Part I. The relations between the concentrations in

Table 1. *Using water containing diacetyl*

No. of run	Vacuum no. 1 stage (in. Hg)	Vacuum no. 2 stage (in. Hg)	Maximum pressure and temp. no. 2 stage p.s.i.g.	Maximum pressure and temp. no. 2 stage ° F.	Maximum temp. no. 1 stage (° F.)	Condenser steam: lb. / lb. water = V/LQ	Diacetyl (p.p.m.) Leaving 1st stage x_2	Diacetyl (p.p.m.) Leaving 2nd stage x_0
Type I. Initial p.p.m. = 8·0. Feed temp. 60° F. Approximate water flow 1000 lb./hr. Bomb valve slack.								
A1	4·5	3·0	Not measured		195	0·038	6·3	0·9
B1	4·5	3·0			201	0·075	2·9	0·6
C1	4·3	3·0			201	0·105	2·1	0·4
D1	4·0	2·5			201	0·150	2·0	0·3
E1	4·0	2·0			202	0·189	1·8	0·4
F1	4·5	2·0			202	0·240	1·6	0·3
G1	5·3	1·8			202	0·294	1·5	0·1*
Type I. Initial p.p.m. = 9·6. Feed temp. 60° F. Approximate water flow 1000 lb./hr. Bomb valve slack.								
A2	6·8	3·5	—	210	200	0·075	4·9	0·7
B2	4·8	3·5	—	212	201	0·107	3·2	0·5
C2	4·8	3·3	0·5	212	201·5	0·135	2·0	0·3
D2	4·8	3·0	1·0	212	202	0·178	1·8	0·3*
E2	5·0	3·0	1·5	214	202	0·216	1·7	0·3*
F2	5·5	1·5	3·0	220	204	0·343	1·2	0·3*
G2	4·4	3·0	0	205	198	0·038	5·9	1·0
H2	4·0	3·0	0	210	202	0·078	3·3	0·4
Type II. Initial p.p.m. = 11·0. Feed temp. 60° F. Approximate water flow 1000 lb./hr. Bomb valve slack.								
A3	Not	4·0	2·5	80	207	0·0085	11 +	2·7
B3	measured	5·0	4·0	200	209	0·102	4·2	0·3
C3		4·5	3·5	200	208	0·049	7·4	0·7
D3		6·0	4·5	203	210	0·165	2·8	0·3
E3		7·0	4·5	204	210	0·206	2·3	0·3
F3		4·0	3·0	198	208	0·034	8·5	0·9
G3		5·0	2·8	184	208	0·012	11 +	2·1

The values marked 11 + in run 3 indicate values considerably greater than 11·0 p.p.m. but were not measured.

Table 2. *Using water containing acetoin*

No. of run	Vacuum no. 1 stage (in. Hg)	Vacuum no. 2 stage (in. Hg)	Maximum pressure and temp. no. 2 stage p.s.i.g.	Maximum pressure and temp. no. 2 stage ° F.	Maximum temp. no. 1 stage (° F.)	Condenser steam: lb. / lb. water out = V/LQ	Acetoin (p.p.m.) Leaving 1st stage x_2	Acetoin (p.p.m.) Leaving 2nd stage x_0
Type I. Initial p.p.m. = 10·0. Feed temp. 60° F. Approximate water flow 1000 lb./hr. Bomb valve slack.								
J2	4·0	2·5	0·0	210	196	0·038	9·9	8·2
K2	3·8	2·5	0·0	212	203	0·075	9·8	7·6
L2	4·0	2·5	0·5	212	203	0·113	9·5	7·1
M2	3·8	2·5	1·0	214	203	0·160	9·2	6·3
N2	5·0	2·0	2·0	216	203	0·225	8·4	5·5
O2	5·5	0·0	3·6	220	205	0·41	6·8	4·5
Type I. Initial p.p.m. = 9·4. Feed temp. 60° F. Approximate water flow 1000 lb./hr. Bomb valve normal for runs B–E. Bomb valve light for run A.								
A4	1·0	14·0	1·0	212	206	0·167	9·4	6·5
B4	3·3	9·0	0·5	212	202	0·175	8·9	6·6
C4	3·0	9·0	0·0	210	201	0·094	9·4	7·4
D4	2·5	10·0	1·0	212	203	0·248	8·4	5·8
E4	1·5	16·5	4·5	225	200	0·590	7·1	3·9

the outgoing steam and water were calculated also for the case of the Vacreator section, and the points obtained in this way were found to be closely scattered along the equilibrium curve obtained from the transfer in the piece of pipe. Thus it could be concluded that the Vacreator stage of the experimental unit reached approximate equilibrium, confirming the conclusions reached by McDowall, and on this basis the curves of Fig. 9

were computed by the graphical method illustrated in Fig. 7. For comparison the appropriate experimental points are plotted also.

The figures confirm the large steam saving that is possible by the use of counterflow methods. For instance, in run G2 the diacetyl content of water was reduced from 9·6 to 1·0 p.p.m. by the use of 0·038 lb. of steam/lb. of liquid leaving. At 18% dilution this represents a deodorizing steam consumption of 0·045 lb./lb. incoming water which is only one-tenth of the deodorizing steam which was used for a similar diacetyl reduction for single contact (see Part I). To the deodorizing steam must be added the steam required for heating, equal to about 0·15 lb./lb., and must be taken into account when assessing the overall economy of either of the systems.

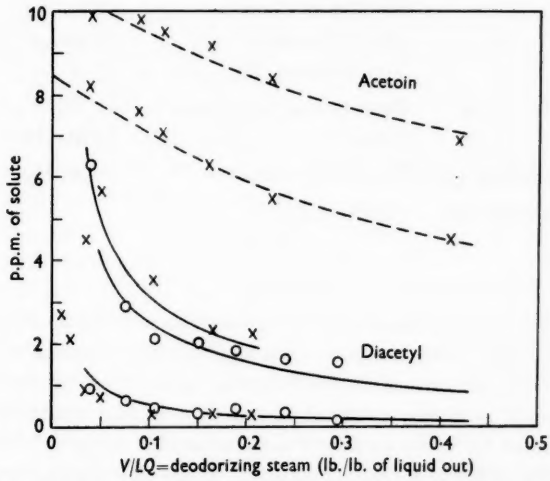

Fig. 9. Comparison of calculated and experimental results for small converted Tandem Vacreator. Dotted lines for acetoin as reference substance in run 2. Full lines for diacetyl as reference substance. Crosses for run 3, circles for run 1. Top line for residual solute at end of first unit. Bottom dotted line for final concentration.

For acetoin the saving in steam is not so pronounced as for the more volatile diacetyl, but the gain is still considerable. Probably a taint with a volatility as low as that for acetoin represents an extreme case, since the degree of taint removal which takes place in a single or double contact system for a taint of such low volatility is not great, and is little affected by steam stripping in any system so far used. The effect of reflux is shown by the high values of x_2 in the tables.

A Tandem, M type Vacreator, in a commercial factory was converted in a manner similar to Fig. 8, and runs were made with water containing diacetyl and also runs were made with cream. The diacetyl in water results were similar to those presented in Table 1. No trouble was experienced in the processing of cream.

The first fully commercial installations of multiple contact counterflow operation of a Vacreator were made by connecting a Triple Vacreator as shown in Fig. 3. Two factories were selected in large butter-producing areas, one being in the Waikato district, the other in Northland. In each case the counterflow connexion was attached to a short extension piece placed on the top of the mixing vessel.

The units were operated with about half the deodorizing steam usually used. Although the steam demand was 2000 lb./hr. less and the water demand 20,000 lb./hr. less, the butter grading was excellent, and did in fact give rise to considerable praise. Vacreator operation was more stable and quieter than usual. No attempt has been made as yet to find out how much further the steam can be reduced without impairing the grade of the resulting butter. At the time of writing other New Zealand factories are converting Triple Vacreators to the system described above.

No attempt has been made to find the best operating conditions, but at present commercial operation is approximately as follows:

No. 1 unit	Pasteurizing temperature	190° F.
	Condenser vacuum	19 in. Hg
	Water pressure	65 p.s.i.g.
	Temperature rise	80° F.
No. 2 unit	Pasteurizing temperature	215° F.
	Vacuum	12 in. Hg
Cooling unit	Condenser vacuum	27 in. Hg
Cream flow	12,000 lb./hr.	

SUMMARY

The considerable increase in efficiency of taint removal which can be attained by counterflow methods is shown by the extension of the algebraic and graphical methods of Part I and confirmed by experiments with the reference substances diacetyl and acetoin in water. The treatment is mainly confined to multiple contact counterflow, and several methods are suggested by which existing Vacreator equipment may be used for counterflow connexion. The effect of a feed temperature below the temperature existing in the contacting vessels is shown to increase the amount of taint removal per pound of stripping steam used. Comparative curves are given for total steam consumption for systems preheating to various temperatures with waste heat.

Results are given for experiments with a converted small size Tandem Vacreator. For diacetyl, the stripping steam used was one-tenth of that for a single contact process over the same range of diacetyl removal. Acetoin, being less volatile than diacetyl, did not show the same large steam saving.

The author wishes to thank Mr P. Halligan, Manager of the Cheltenhan Co-operative Dairy Co. for his assistance in the installation of the first trial unit, and Messrs R. Goodman and J. Stevenson, Managers of the Te Awamutu Co-operative Dairy Co. and the Maungatapere Co-operative Dairy Co., respectively, for their ready willingness in the conversion of the Vacreators in their factories.

The author also wishes to thank Dr F. H. McDowall of the Dairy Research Institute (N.Z.) for helpful discussions, and Mr E. R. Elley of the same Institute for his care and attention to the diacetyl and acetoin determinations.

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NOMENCLATURE

F = stripping factor mV/L dimensionless, F_1 for first vessel and F_2 for second vessel.
G = inflow of steam to any system (lb./hr.).
L = inflow of liquid to any system (lb./hr.).
m = equilibrium constant = y_e/x_e at any point.
N = number of contacts in a multi-contact system.
Q = dilution factor = $1+S/L$, dimensionless.
S = weight of steam condensed and adding to the liquid flow (lb./hr.).
V = outflow of steam in any system (lb./hr.).
x = concentration of taint in liquid at any point (lb./lb.).
x_1 = concentration of taint in incoming liquid (lb./lb.).
x_2 = concentration of taint in liquid leaving first vessel and entering second vessel (lb./lb.).
x_0 = concentration of taint in liquid leaving the system (lb./lb.).
x_e = concentration of taint in liquid which is in equilibrium with y_e (lb./lb.).
y = concentration of taint in steam at any point (lb./lb.).
y_1 = concentration of taint in steam entering a system (lb./lb.).
y_2 = concentration of taint in steam leaving a system (lb./lb.).
y_0 = concentration of taint in steam leaving a vessel and in contact with concentration x_0 (lb./lb.).
y_e = concentration of taint in steam which is in equilibrium with x_e for any value of x_e (lb./lb.).

APPENDIX

Final taint concentration for double contact counterflow

(The following analysis is based on equations derived in Part I)

Vessel 2
$$\frac{x_0}{x_2}=\frac{1}{1+F_2}, \tag{1}$$

where
$$F_2=\frac{m_2 G}{LQ} \quad \text{and} \quad Q=\left(1+\frac{S}{L}\right);$$

and
$$y_0=\frac{x_2-x_0}{G/LQ}. \tag{2}$$

The temperature of the feed LQ entering vessel 2 is assumed at the saturation temperature of the steam so that no condensation takes place in this vessel.

Vessel 1
$$\frac{x_2}{x_1}=\frac{1+\dfrac{Gy_0}{Lx_1}}{Q+F_1}, \tag{3}$$

where $F_1=\frac{m_1V}{L}$. Inserting the value of y_0 from equation (2) into (3) and re-arranging

$$\frac{x_2}{x_1}=\frac{1-Q\frac{x_0}{x_1}}{F_1}. \tag{4}$$

Since $\frac{x_0}{x_1}=\frac{x_2}{x_1}\frac{x_0}{x_2}$, multiplying equations (1) and (4) gives $\frac{x_0}{x_1}=\frac{1-Q\frac{x_0}{x_1}}{F_1(1+F_2)}$ and upon re-arrangement

$$\frac{x_0}{x_1}=\frac{1}{Q+F_1(1+F_2)}. \tag{5}$$

Case of no condensation

If the feed L to the system is at the saturation temperature of the contacting steam there is no condensation (and $Q=1$) within the vessels and $G=V$. Assuming equal values for the equilibrium constants in each vessel $m=m_1=m_2$ and thus $F=F_1=F_2$.

Equation (5) becomes

$$\frac{x_0}{x_1}=\frac{1}{1+F+F^2}=\frac{F-1}{F^3-1}.$$

A similar procedure will show that for N vessels in counterflow arrangement

$$\frac{x_0}{x_1}=\frac{F-1}{F^{N+1}-1}.$$

In practice, vessel 2 will be at a slightly greater pressure and temperature than vessel 1, but this small effect is neglected in this treatment of the problem.

Case of no deodorizing steam, $V=0$

If no steam leaves the system then no taint is removed. The concentration of taint in the outgoing liquid is x_0 equal to x_1/Q since dilution of the incoming liquid takes place.

Considering vessel 2,

$$\frac{x_2}{x_0}=1+F=1+\frac{m_2G}{LQ},$$

but $x_0=x_1/Q$; therefore $\frac{x_2}{x_1}=\frac{1+(mS/LQ)}{Q}$ since $G=S$ when $V=0$.

Thus when $m=Q$, $x_2=x_1$ and, in general, the value of x_2 will depend on the value of m being large, when m is large.

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564. THE PROPERTIES OF NEW ZEALAND BUTTERS AND BUTTERFATS

IV.* REFRACTIVE INDEX AND DENSITY OF THE BUTTERFAT

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(With 3 Figures)

The refractive index and the density of butterfat are physical properties of considerable importance in dairy analytical work. Refractive index determination is used as an aid to the detection of adulteration of butterfat with other fats; and uniformity in the density of butterfat in all milk-producing countries is implied by the universal use of volumetric methods for the estimation of fat in dairy products. Although the physical properties of butterfat have received less systematic study than the chemical properties, there are available, especially in the earlier literature, a number of reports in which figures for refractive index and density are quoted; but in these reports there is no reference to New Zealand butterfat.

SOURCE AND PREPARATION OF SAMPLES

The butterfats were prepared from samples of fresh butter obtained at 8-week intervals, over the period August 1945 to December 1948 (41 months), from twenty butter factories representing the chief buttermaking and cheesemaking districts of New Zealand.† The locality of these factories is given in Table 1.

The butters were melted and the butterfat layer was decanted and filtered at 60° C.

Table 1. *Locality distribution of butter factories supplying samples for analysis*

Locality	No. of factories
North Island	
North Auckland (factories north of the city of Auckland)	4
South Auckland (including Franklin County, the Waikato, Thames Valley, and the King Country)	5
Bay of Plenty	2
Gisborne	1
Hawkes Bay	1
Taranaki	2
Wellington (including Manawatu, Wairarapa and Pahiatua)	3
South Island	
Canterbury	1
Southland	1

METHODS

Refractive index (n^{40}) was measured with a compensated Zeiss-Abbé refractometer at 40° C. The specific gravity of the fat was measured with a 25 ml. specific gravity bottle at 60° C.‡ against water at 60° C., observing all precautions to ensure the accuracy of the

* Seasonal variations in iodine, Reichert and saponification values and in softening points of New Zealand butterfats have been studied already by Cox & McDowall (1).

† The samples were required primarily for the estimation of the vitamin A potency of New Zealand butter (2).

‡ The temperature of 60° C. was chosen so that the possible effects of the variations in density of the butterfat on the readings for the Babcock and Gerber tests—read at 60° C. and 65° C. respectively—might be more clearly apparent.

determination (see *British Standard* 684, 1950, p. 59; and *Official Methods of Analysis of the Association of Official Agricultural Chemists*, 1950, p. 427). The density of the butterfat in g./ml. at 60° C. was calculated by the use of the formula:

$$\text{density } (D60) = \text{specific gravity } {}^{60°}_{60°} \times \text{density } 60° \text{ of water.}$$

RESULTS

(1) *Range of variation of results.* The maximum and minimum results for refractive index and for density over the whole period of sampling are given in Table 2, together with some results for European and United States butterfats. The values for refractive index are within the range quoted by Elsdon & Walker(3) for European butterfats. Maximum and minimum density results are higher than those reported by Jenness, Herreid, Caulfield, Burgwald, Jack & Tucker(4) for United States butterfats drawn from a large area, but lower than those of Miller, Coulter & Combs(5) for Minnesota mixed herd butterfats in the late autumn and winter.

Table 2. *Maximum and minimum results for refractive index and for density*

	Refractive index (n^{40})		Density (D 60)	
Source of butterfat	Minimum	Maximum	Minimum	Maximum
New Zealand (present investigation)	1·4535	1·4571	0·8885	0·8916
Europe (Elsdon & Walker(3))	1·4522	1·4580	—	—
United States (Jenness *et al.*(4))	—	—	0·8867	0·8910
United States (Miller *et al.*(5))	—	—	0·8900	0·8940

The maximum range of variation in density of New Zealand butterfat would be equivalent to a variation of only ±0·015% in the Babcock and Gerber readings for a milk of 4·5% fat content. This is in accord with the conclusions of Jenness & Herreid(6) that variations in the density of the pure milk fat have no appreciable effect on the accuracy of the Babcock test.

(2) *Mean results for each sampling date.* The refractive index and the density results were fairly uniform for any one sampling date for the butterfats from the eighteen North Island factories. The results for the butterfats from the two South Island factories were correspondingly uniform. The mean values for North Island and South Island butterfats for each sampling date are presented in Table 3 and are plotted in Fig. 1. Standard deviations were calculated for the eighteen New Zealand butterfats for each sampling date. Both for refractive index and density they ranged from 0·0001 to 0·0004 (Table 3). The average divergence between the results for the two South Island butterfats was: n^{40}, 0·0002; D60, 0·0003.

There were distinct seasonal changes in refractive index, although the pattern of the change was not always consistent from year to year (see Fig. 1). Results for both North Island and South Island butterfats were at a maximum in the autumn months of April and May (the one exception was in 1946 when the South Island maximum occurred in October) and at a minimum in the late spring and summer (November, December, January). In some years, however, the fall from maximum to minimum was not uniform but was arrested by a temporary rise in September–October (see Fig. 1, and compare with Fig. 1 (iodine values) in Part I of this series(1)). The minimum refractive index results, like the minimum iodine values, of New Zealand butterfat(1) occur at the season

of the year when the refractive index results for Northern European butterfats are at their maximum (3, 7, 8, 9, 10).

The density results also showed a seasonal change which in comparison with that for the refractive index was very regular from year to year (see Fig. 2). Maximum density results for both the North Island and South Island butterfats were found each year in the late spring period, viz. October or November, and minimum results in the autumn (March, April or May).

Table 3. *Mean results for the refractive index and the density of the North Island and South Island butterfats for each sampling date*

	Refractive index (n^{40})			Density ($D60$)		
	North Island		South Island	North Island		South Island
Date of sampling	Mean	S.D.*	(mean)	Mean	S.D.*	(mean)
6. viii. 45	1·4555	0·0004	1·4551	0·8898	0·0002	0·8899
1. x. 45	1·4559	0·0004	1·4558	0·8907	0·0002	0·8905
26. xi. 45	1·4540	0·0002	1·4540	0·8909	0·0002	0·8911
21. i. 46	1·4547	0·0004	1·4546	0·8901	0·0003	0·8905
18. iii. 46	1·4559	0·0003	1·4556	0·8893	0·0002	0·8897
13. v. 46	1·4565	0·0004	1·4560	0·8894	0·0003	0·8896
8. vii. 46	1·4559	0·0002	1·4551	0·8897	0·0003	0·8900
2. ix. 46	1·4559	0·0001	1·4560	0·8904	0·0002	0·8902
28. x. 46	1·4555	0·0003	1·4561	0·8910	0·0003	0·8907
6. i. 47	1·4550	0·0001	1·4549	0·8906	0·0004	0·8901
3. iii. 47	1·4552	0·0002	1·4550	0·8901	0·0003	0·8902
28. iv. 47	1·4560	0·0003	1·4559	0·8893	0·0004	0·8893
23. vi. 47	1·4557	0·0002	1·4549	0·8893	0·0003	0·8897
18. viii. 47	1·4548	0·0001	1·4548	0·8904	0·0001	0·8903
13. x. 47	1·4542	0·0001	1·4544	0·8912	0·0002	0·8909
8. xii. 47	1·4541	0·0003	1·4538	0·8908	0·0002	0·8910
2. ii. 48	1·4545	0·0002	1·4545	0·8902	0·0002	0·8905
29. iii. 48	1·4549	0·0002	1·4548	0·8894	0·0002	0·8901
24. v. 48	1·4560	0·0001	1·4553	0·8891	0·0004	0·8900
19. vii. 48	1·4559	0·0002	1·4551	0·8900	0·0003	0·8902
13. ix. 48	1·4551	0·0001	1·4551	0·8908	0·0003	—
8. xi. 48	1·4549	0·0002	1·4550	0·8913	0·0002	0·8916

* S.D. is standard deviation of a single factory sample.

There are few references in the literature to variation in the density of butterfat with variation in feeding conditions or with season of the year.* Werenskiold (10) found a higher specific gravity for Norwegian butterfat in winter than in summer. Groh (11) noted that the specific gravity of Northern European butterfat remained constant during the transition from winter to summer feeding. Jenness *et al.* (4) concluded that the density of purified milk fat in the United States was relatively constant and was not markedly affected by breed, season or feed. Uniform seasonal trends in density similar to those reported above for New Zealand butterfats apparently have not been noted elsewhere.

(3) *Grouping of results according to month of sampling.* A uniform grouping of the results according to month of sampling was not possible owing to the effect of the long intervals between sampling on the evenness of distribution of the sampling dates according to months. Thus there was only one sampling date for February, April, June and December, compared with three for March and October and two for the remaining months (Table 4). On the assumption that the figures for each month are reasonably uniform

* Because of the seasonal nature of dairying for butter manufacture in New Zealand, effects of stage of lactation on butterfat properties are superimposed on the effects of feeding conditions (1, 2).

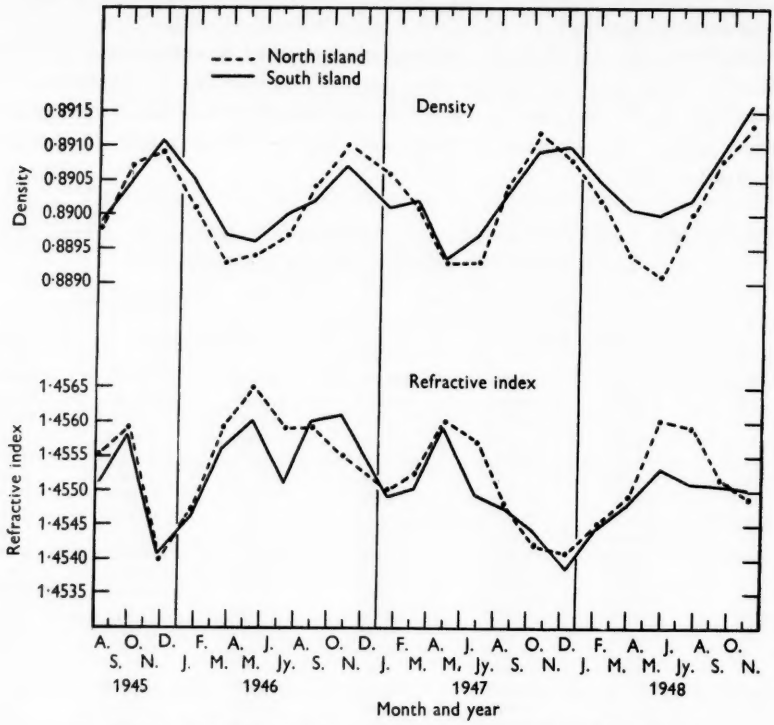

Fig. 1. Mean results for the refractive index and density of the North Island and the South Island butterfats for each sampling date.

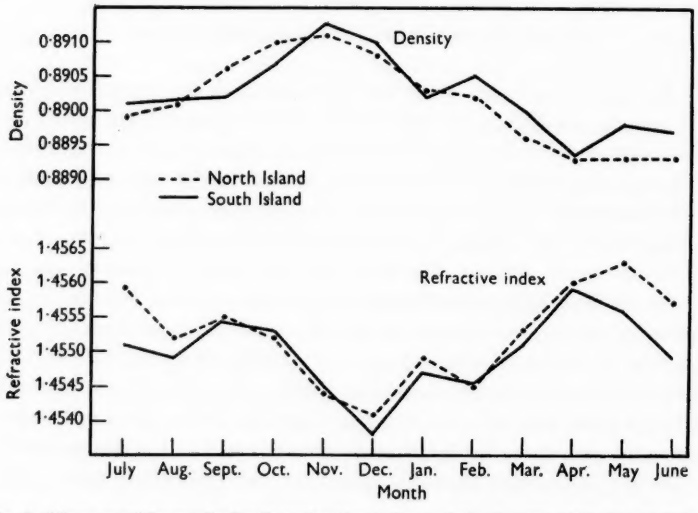

Fig. 2. Mean monthly results for the refractive index and the density of the North Island and the South Island butterfats.

from year to year* the results for refractive index and density for each sample over the 41-month period were grouped according to month of sampling and irrespective of year. The mean monthly results for North Island and South Island butterfats are given in Table 4.

Table 4. *Mean monthly results for the refractive index and the density of the North Island and South Island butterfats*

		Refractive index (n^{40})				Density ($D60$)			
		North Island		South Island		North Island		South Island	
Month	No. of sampling days	Mean	S.D.*	Mean	S.D.*	Mean	S.D.*	Mean	S.D.*
July	2	1·4559	0·0002	1·4551	0·0001	0·8899	0·0004	0·8901	0·0003
Aug.	2	1·4552	0·0004	1·4549	0·0002	0·8901	0·0003	0·8901	0·0002
Sept.	2	1·4555	0·0004	1·4555	0·0005	0·8906	0·0003	0·8902	—
Oct.	3	1·4552	0·0008	1·4553	0·0009	0·8910	0·0003	0·8907	0·0003
Nov.	2	1·4544	0·0005	1·4545	0·0006	0·8911	0·0003	0·8913	0·0003
Dec.	1	1·4541	0·0003	1·4538	—	0·8908	0·0003	0·8910	—
Jan.	2	1·4549	0·0003	1·4547	0·0004	0·8903	0·0004	0·8902	0·0003
Feb.	1	1·4545	0·0002	1·4545	—	0·8902	0·0002	0·8905	—
Mar.	3	1·4553	0·0005	1·4551	0·0005	0·8896	0·0004	0·8900	0·0004
Apr.	1	1·4560	0·0003	1·4559	—	0·8893	0·0004	0·8893	—
May	2	1·4563	0·0004	1·4556	0·0004	0·8893	0·0004	0·8898	0·0003
June	1	1·4557	0·0002	1·4549	—	0·8893	0·0003	0·8897	—

* S.D. is the standard deviation of a single factory sample.

The mean monthly results over the 12-month period July to June, which represents, fairly closely, the lactational year in New Zealand, are plotted in Fig. 2, where the seasonal trends referred to above are clearly shown. North Island and South Island means agree fairly closely except in the winter months of May, June and July when there is a divergence in both refractive index and density results. A similar divergence between the properties of North Island and South Island butterfats has already been reported for iodine, saponification and Reichert values (1). The explanation probably lies in the different feeding conditions in winter (1). In the North Island supplementary crops consist mainly of hay and silage, but in the South Island, with less pasture available, the feeding of root crops is general.

(4) *Weighted mean results.* The refractive index and density results for butterfat from all twenty factories for any one month were fairly uniform; and as the locality of these factories represented, fairly closely, the distribution of butter production throughout New Zealand, an allowance for district variations in the volume of butter produced each month was considered unnecessary in the calculation of weighted averages. Weighted means for the whole period of the investigation therefore were calculated from the mean monthly results for refractive index and for density, and the average monthly overall production of butter expressed as a percentage of the average annual butter production in New Zealand. The weighted mean results were: refractive index, 1·45492; density, 0·89045.

(5) *Interrelationship of butterfat properties.* A comparison of the mean monthly results for all twenty factories with the results for certain butterfat properties reported by Cox & McDowall (1) show certain marked similarities (see Fig. 3). Thus the curves for refractive index and for iodine value are very similar, and seasonal changes in density are paralleled

* The assumption is not entirely justified, e.g. in the month of October the standard deviations for refractive index results from three sampling dates are: North Island 0·0008, South Island 0·0009.

by the changes in saponification value and, to a lesser extent, by the changes in Reichert value (Fig. 3).

In the present investigation the iodine value, the refractive index, the density, the saponification value and the Reichert value were determined on all butterfats from

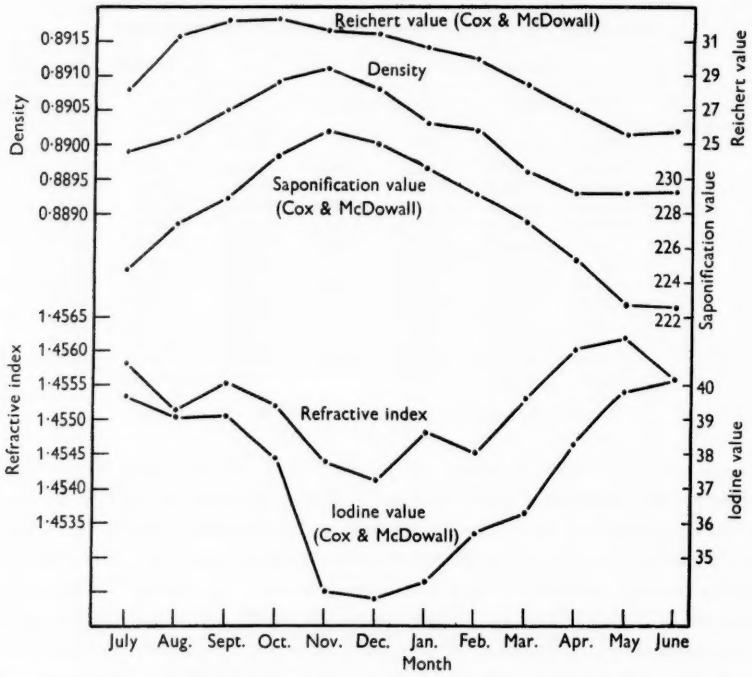

Fig. 3. Mean monthly results for the refractive index and the density of all the butterfat samples. Also weighted mean monthly iodine, Reichert and saponification values (from Cox & McDowall(11)).

Table 5. *Correlation coefficients and regression equations for some butterfat properties*

Butterfat properties	Correlation coefficients based on 140 pairs of observations	Regression equations	Standard deviation from regression line
Iodine value (I) and refractive index (R)	$+0{\cdot}826 \pm 0{\cdot}048$**	$I = +2820{\cdot}7R - 4068{\cdot}2$	(I) 1·67
Density (D) and saponification value (S)	$+0{\cdot}943 \pm 0{\cdot}028$**	$D = +0{\cdot}0001753S + 0{\cdot}8499$	(D) 0·00022
Density (D) and Reichert value (Rt)	$+0{\cdot}893 \pm 0{\cdot}038$**	$D = +0{\cdot}0002488Rt + 0{\cdot}8827$	(D) 0·00029

** Highly significant.

August 1945 to July 1946,* a period which included seven sampling dates and a total of 140 butterfats. Correlation coefficients between the various sets of results, and the corresponding regression equations, are shown in Table 5. It must be stressed that regression equations such as these may apply only to the series of observations on which

* Unfortunately the determination of iodine, saponification and Reichert values was discontinued after this date.

they are based, as Janse(12) and Winther(13) have already pointed out, and will not necessarily apply to the results reported in other years.

The correlation between iodine value and refractive index is not unexpected in view of the work of earlier investigators who have found that the refractive index was influenced mainly by the content of unsaturated fatty acids in the butterfat(14). Later, the relationship was confirmed for Swedish butterfat (correlation coefficient, +0·953)(7) and for Norwegian butterfat (correlation coefficient, +0·987)(15).

The high degree of correlation between density and saponification value and between density and Reichert value suggests that all three properties are closely related. A study of the table, compiled by Richmond(16), of mean results for the properties (including Reichert and saponification values and specific gravity 37·8°/37·8° C.) of over 300 butterfats show that this relationship applies also to butterfats from English butters.

Hansen & Shorland(17), working on New Zealand butterfat, have also pointed out the close relationship that exists between saponification value and Reichert value which itself is correlated with the content of C_4–C_{12} saturated acids(17). Increases in the molecular weight of the fatty acids comprising the mixed glycerides of butterfat obviously will be reflected in decreasing saponification values and also in decreasing densities if the general rule for fats and oils applies(18). It is also an accepted rule that the specific gravity varies with the iodine value; and a similar variation for butterfat has been assumed, from theoretical considerations, by Dutch workers(19). In New Zealand butterfat, however, increases in the content of C_4–C_{12} saturated acids are reflected in compensating decreases in the content of C_{18} unsaturated acids but in slight changes only in the quantity of palmitic acid(17). Changes in both the saponification values and the density will therefore be governed almost entirely by variations in the content of the lower saturated fatty acids.

SUMMARY

1. The refractive index and density of butterfats were determined for butters forwarded by twenty factories at 8-week intervals over a period of 3 years. The refractive index (n^{40}) ranged from 1·4535 to 1·4571, with mean weighted value of 1·45492. The density ($D60$) ranged from 0·8885 to 0·8916, with mean weighted value of 0·89045.

2. Minimum refractive index results were found in the summer butterfats and maximum results in the winter butterfats, i.e. at opposite seasons to the occurrence of the minimum and maximum in Northern European butterfats. Maximum density results were found in late spring butterfats and minimum results in autumn butterfats.

3. The mean monthly results for refractive index and for density of North Island and South Island butterfats showed an appreciable divergence only during the winter months.

4. The correlations between refractive index and iodine value, between density and saponification value, and between density and Reichert value, on 140 butterfats sampled over 1 year were all highly significant.

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619.2

REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION E. DISEASES OF DAIRY CATTLE

CONTENTS*

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I. MASTITIS

A. *Treatment*

In the past two years further important work on the treatment of mastitis caused by *Streptococcus agalactiae* has been published. Ford & Wilson(1) found no tendency for *Str. agalactiae* to develop resistance to penicillin as a result of intramammary treatment with this drug. Using the ditch-plate technique, Aynsley(2) tested the sensitivity to penicillin of organisms isolated from cases of bovine mastitis. The results obtained in a series of cultures showed no evidence that the widespread use of penicillin was resulting in increased resistance on the part of streptococci causing bovine mastitis, and no highly resistant strains of staphylococci were isolated. Edwards(3) considers that quarters infected with *Str. agalactiae* which failed to clear by the use of intramammary penicillin, were in many instances cases of reinfection, from teat sores, milkers' hands or udder cloths. Only a few cases seemed to be really resistant to treatment and these might well be explained by a close study of the glandular structure. Edwards gives a simple procedure for the control of *Str. agalactiae* in a herd. This consists of taking two milk samples from each cow at an interval of 1 week, and treating all infected cows and dry cows with two doses of 100,000 units of penicillin at a 3-day interval. For a week following the treatment, the milkers should disinfect their hands with an antiseptic cream at milking time. Hand stripping is dispensed with, and the udders are washed with separate sterile cloths using a suitable disinfectant in the washing water. All animals are retested in 6 weeks and all infected animals retreated. A further test is made at 12 weeks, and at this time it may be necessary to dispose of any cows which are resistant to treatment. Further tests are made at 6-monthly intervals or longer. Wilson(4) points out that in England the incidence of *Str. agalactiae* in the udder is still high, though it does not always cause mastitis. Because *Str. agalactiae* is an obligatory parasite and is penicillin-sensitive, eradication from a herd is a practical proposition. He gives three reasons for difficulty in herd eradication of *Str. agalactiae*: (1) the presence in the herd of cows resistant to treatment, (2) the presence of teat sores causing reinfection, and (3) infection of the hands of the milkers with the organism. Neave, Philips & Mattick(5) discuss the difficulty of keeping herds free from *Str. agalactiae* for long periods, but the herds they studied were large and were under one management with interchange of milkers. They advocate that hand-stripping of cows should be abolished. It is interesting to note, however, that later Neave, Higgs, Simpkin, Oliver & Dodd(6), in a controlled experiment on two herds, found that the method of stripping had little influence on the infection rate of *Str. agalactiae*. Details of a mastitis control programme in New York State are given by

* The years covered by this review were mainly 1952 and 1953.

Payne & Elliott(7). Quarter samples are taken from a herd and the general milking management checked. Treatment is given as soon as possible, usually with 300,000 units of penicillin per quarter. The authors give details of the results for five herds, two of which were cleared of *Str. agalactiae* infection.

In a study of the *in vitro* and *in vivo* action of certain antibiotics, Edwards(8) found that there was no strict correlation between *in vitro* sensitivity of organisms and the susceptibility *in vivo* when a standard dose of 100×10^6 organisms/ml. was injected into mice. He suggested that the *in vivo* test should be used to confirm the selection of an antibiotic for therapeutic use in clinical infection. Only penicillin possessed a marked bacteriological activity, which was especially marked against Gram-positive organisms. Aureomycin compared unfavourably with penicillin *in vitro* when judged by its bacteriocidal effect, but *in vivo* it gave better protection against streptococci and staphylococci and good protection against *Bacterium coli*. Streptomycin was poor against staphylococci *in vitro*, whereas *in vivo* it was better than penicillin. Edds, Roepke & Bieter(9) have studied *in vitro* the effect of penicillin, streptomycin, aureomycin, terramycin and sulphisoxazole alone, or in combination, on micrococci isolated from the bovine udder. Combinations of pairs of drugs were frequently more effective than when the drugs were given separately, exceptions being penicillin with terramycin and penicillin with aureomycin. The addition of sulphisoxazole to an antibiotic resulted in a general increase of activity against most strains of micrococci. Edwards & Haskins(10) have determined the antibiotic levels in blood and milk of cows following parenteral and intramammary injection. Intravenous administration of penicillin, aureomycin and streptomycin and intramuscular administration of penicillin and streptomycin all gave similar results; the blood level fell rapidly and the milk level was at first maintained and then also fell rapidly. After intramuscular administration of aureomycin, however, the blood and milk levels were both retained for 48 hr. Neither calcium penicillin in beeswax and arachis oil nor procaine penicillin with aluminium stearate were consistently present 48 hr. after intramammary infusion. In a cow giving 25 lb. milk per day traces of aureomycin could be recovered for 6 days after intramammary infusion of 210 mg. of this antibiotic; 250 mg. dihydrostreptomycin was not retained in the udder after 2 days even in low yielding cows. Uvarov & Muggleton(11) have shown that intramammary injection of 300,000 units of procaine penicillin in mineral oil with aluminium stearate added, gave, up to a period of 14 days, levels sufficient to control mastitis caused by most penicillin sensitive organisms. Schipper & Petersen(12) found that aureomycin given intravenously reached the same concentration in the milk as in the blood in 2 hr., whereas, following intramammary infusion, never more than 1 μg./ml. could be found in the blood, regardless of the amount infused. They therefore suggest that the intramammary route is preferable for this drug.

Treatment trials have been mainly confined to the use of antibiotics other than penicillin. Murphy & Stuart(13), however, have attempted treatment of *Str. agalactiae* mastitis by the intramuscular administration of penicillin. They used three levels of dosage and only the highest level, which consisted of 42 million units spread over a period of 7 days, was successful. Schalm & Woods(14) have tried the effect of massive doses of penicillin and dihydrostreptomycin employed singly or in combination on *Staphylococcus pyogenes* infections. Doses of up to one million units of penicillin and up to 1 g. dihydrostreptomycin were used for each intramammary injection. They concluded that the two drugs combined gave better results than penicillin alone, especially in lactating glands. Comparing the effect of intramammary penicillin with streptomycin and dihydrostreptomycin, Simon, Spencer & Schmidt(15) found that the latter drugs were as good as penicillin against *Str. agalactiae*, and that in addition, dihydrostreptomycin was effective against *Bact. coli*. Schalm & Woods(16) have discussed the characteristics of coliform mastitis in a large dairy herd from which *Str. agalactiae* had been eradicated and infection with *Staph. pyogenes* had been reduced from 52 to 6%. Several types of coliform organisms were incriminated. Treatment with dihydrostreptomycin in general proved satisfactory in removing the infection and returning the cows to normal milk production.

Trials with intramammary infusion of aureomycin have been made by Drury(17). Of twenty-

four cows with staphylococcal mastitis sixteen responded to treatment, and of twenty-three cows with streptococcal mastitis seventeen responded to treatment. It is interesting to note that four of the six quarters with streptococcal mastitis, which were resistant to treatment, responded to penicillin. Kästli & Baumgartner(18) found that the intramammary injection of aureomycin in oil caused irritation of the gland for a period of 3–15 days. Treatment, however, destroyed the organisms in nine out of ten cases of staphylococcal mastitis and in twenty out of twenty-one cases of mastitis caused by *Str. agalactiae*. Injection of up to 5 g. neomycin sulphate into the udders of healthy cows was found by Drury(19) to cause little or no irritation as diagnosed by leucocyte count, direct microscopic examination, methylene blue and other indirect tests. He claims that the results of treating 405 cases of mastitis indicated that this drug was effective against streptococci, staphylococci, coliform organisms and a yeast. Simon, Radivojevic & Reese(20), however, state that their work on neomycin sulphate does not confirm Drury's reports. They found that this drug was not as effective as penicillin. The fact that Drury used the microscopic examination of incubated samples as his method of diagnosis may be one reason for these conflicting reports. Some success in the use of neomycin sulphate is reported by Tucker & Johnson(21) in that they obtained bacteriological and clinical cures of a total of seventeen out of forty-three cases of mastitis associated with pseudomonas. They are of the opinion that penicillin is superior to neomycin sulphate against streptococcal mastitis. The use of terramycin in the treatment of streptococcal mastitis has been studied by Simon & Schmidt(22). They found that the drug resembled penicillin in not inciting inflammation in the mammary gland. They carried out two field trials comparing the drug with penicillin. In the first trial, using 30 mg. terramycin, penicillin in the usual dose was more effective, whilst in the second trial using 60 mg.; the results were about equal, but neither were good; penicillin cleared only 56·5% of the cased treated, whereas terramycin cleared 59·4%.

According to Arnandez(23) penetration of a dye or indian ink into an udder, as seen after freezing and slicing the gland, appeared better when hyaluranidase was added. Following the work of Spencer & McNutt, referred to in the previous Review of this series(24), that occlusion of ducts with inflammatory debris is of great importance in the pathogenesis of mastitis, Schipper(25) has tried oxytocin, the hormone responsible for milk ejection, in mastitis therapy. He injected 10–40 units of the drug intravenously after emptying as much milk as possible from the quarter and milked it again. In glands which were very swollen a second dose was given 15–20 min. later and the quarter again milked. He described six quarters in which the milk returned to normal; in four of them no other treatment was given. Of the other two quarters, in addition to the oxytocin treatment, one was infused with penicillin and the other with aureomycin. Rather surprisingly he advocated that milking should be suspended for a period of 18–24 hr. after treatment with an antibiotic. Thomas, Prier, Collopy & Labudovich(26) have tried ephedrine with antibiotics in the treatment of acute mastitis. They state that ephedrine showed a marked activity in reducing congestion and swelling in the affected udder. Rothenberg(27) has described a new intramammary treatment for mastitis caused by organisms which are not susceptible to sulphonamides or antibiotics. It involves the use of a 'mercuriated sterol'. The active ingredients have a tendency to float, which the author considers beneficial since it will enable them to attain and maintain contact with the affected areas. This is false reasoning as many of the smaller ducts in the udder drain upwards. Walker(28) has used a combination of sodium sulphamerazine and sodium sulphamethazine intravenously in eight cases of gangrenous mastitis. Two animals died and six recovered.

B. *Diagnosis*

Streptococci. An evaluation of the CAMP phenomenon(29), using bovine blood in place of sheep blood has been carried out by Murphy, Stuart & Reed(30). Of 322 streptococci classified as *Str. agalactiae* 96·6% produced a CAMP positive reaction; of forty-six classified as *Str. dysgalactiae* none reacted, whereas of 261 classified as *Str. uberis* 14·9% produced a positive reaction. Identification of *Str. uberis* was based solely on its ability to split aesculin; this is not justified. False negative reactions of *Str. agalactiae* occurred in five herds one of which was classified as free from

the organism. False positive reactions of *Str. agalactiae* occurred in twelve of fifteen herds which were truly free from the organism. Hoomand (31) describes a method for carrying out the CAMP test. Several drops of milk are placed on the surface of 5% calf-blood agar and spread radially. Staphylococci which form β-toxin are then inoculated on to the plate by means of a steel stamp which is made in the form of the spokes of a wheel. The inoculum is transferred from a heavily seeded plate which may be used for a large number of inoculations. As commercial companies in the United States have ceased to stock streptococcal antiserum, Bruner & Tucker (32) describe how to prepare such sera in rabbits and how to use them. Moreira Jacob (33) recommends the use of aesculin-blood agar for the identification of streptococci found by the Hotis test.

Staphylococci. Neave *et al.* (5) point out that when enrichment methods, together with solid media selective for streptococci, are used for diagnostic purposes, many staphylococcal infections will undoubtedly be wrongly attributed to streptococci, especially when only one milk sample is examined. Isolating *Staph. pyogenes* from samples taken from two farms, using enrichment methods, Schalm & Woods (34) found different areas of α- and β-haemolysis from the organisms from each farm. They considered that the criteria of pathogenicity should be the ability of the organism to coagulate rabbit plasma, or its ability to produce β-toxin. The same authors (35) report that consecutive herd surveys for *Staph. pyogenes* may reveal considerable variation from test to test, but that the average incidence may exceed 50% of the cows. They consider that the source of *Staph. pyogenes* is essentially other infected mammary glands. Following on the work of Barber & Kuper (36) that phosphatase production closely paralleled coagulase production in numerous strains of staphylococci, Spencer & Lasmanis (37) found that all of ninety-four coagulase positive cultures of *Staph. pyogenes* produced phosphatase, but that seventy-three coagulase negative cultures also produced it. Coagulase positive staphylococci appeared to be more resistant to the action of hypochlorite than coagulase negative staphylococci, and the dipping of teat cups in hypochlorite solution was shown to be ineffective in many cases. After carrying out survival tests of staphylococci, including tests on straw, the authors concluded that under the conditions of the observations, the principal reservoirs for coagulase positive staphylococci, other than the infected mammary gland, are the skin of the teats and the teat cups of the milking machine. If this is true the control of mastitis due to staphylococci should be no more difficult than the control of mastitis due to *Str. agalactiae*.

Levy, Rippon & Williams (38) typed by bacteriophage, coagulase positive staphylococci which had been examined for the production of diffusible toxin, α- and β-haemolysis, fibrolysis and pigment. In strains from human sources they found no significant connexion between phage group and the other properties. Strains of staphylococci from animals seemed to belong to a different biological group from those from human sources.

Cell counts. Using a differential technique to stain the cytoplasmic granules in granular leucocytes, Blackburn & Macadam (39) have studied the cells in bovine milk. They classify the cells into three groups, granular leucocytes, lymphocytes and epithelial cells. Granular leucocytes are the granular cells of the blood; the polymorph, the eosinophil and the basophil. They describe two types of epithelial cells, one vacuolated and one non-vacuolated, both of which are associated with involution, either the involution of late lactation or post-inflammatory involution. The vacuolated type is found in the milk when milking has been suspended or when a duct has been blocked. In an extensive study of staphylococci in six dairy herds, Campbell (40) made total cell counts on a large number of milk samples. The description of his work includes the following figures:

No. of milk samples examined	Organisms present	Average total cell count (cells/ml.)
946	Normal milk	69,000
308	Non-haemolytic staphylococci	245,000
151	Weakly haemolytic staphylococci	960,000
341	Markedly haemolytic staphylococci	1,200,000

The first of these counts may well be regarded as typical of normal milk and the others of milk from udders infected with staphylococci of varying degrees of virulence. In a study of the cell counts of milk from healthy cows, MacLeod & Anderson(41) found that by the middle of the third week of lactation the geometric mean count was 70,000 cells per ml. Slanetz & Bartley(42) do not consider that the 'leucocyte count' can be relied on to indicate the presence of chronic staphylococcal mastitis, since samples taken from such infections of more than a year's standing often contained less than one million cells per ml. MacLeod, Plastridge, Anderson, Gullet & Hale(43) have related the 'leucocyte count' of herd milk to the incidence of mastitis. They found that much of the variation in the average counts of a herd could be explained in the terms of the percentage of mastitis in the herd, and concluded that if the percentage of infected animals in a herd is 40% or more the average count of the herd milk may be expected to be 1,000,000 per ml. or more.

C. *Experimental mastitis*

Mastitis in goats induced by the intramammary injection of *Str. dysgalactiae* is reported by Holman, Smith & Pattison(44) and Pattison & Smith(45). They concluded that the clinical and haematological changes, the alterations in the milk detectable by biochemical tests and the histological changes in the tissues, were all indistinguishable from those produced in goats by *Str. agalactiae* as reported in the previous Review of this series(24). This significant finding suggests that the types of mastitis caused in the cow by these two organisms are indistinguishable.

A pipette for the introduction of minute amounts of bacterial culture into the teat cavity of the bovine udder is described by Murphy(46). With the particular culture medium he used the pipettes were found to deliver from three to fifty-one viable organisms. Using these pipettes, Murphy & Stuart(47) studied the effect of introducing small numbers of *Str. agalactiae* (Cornell 48 strain) directly into the bovine teat cavity. The organisms were injected 1 hr. after the morning milking, and milk samples were drawn just before the evening milking, 9 hr. later. After 9 hr. no organisms were found in three quarters and from 200 to 2000 organisms per ml. in six quarters. After 24 hr. all nine quarters showed 20,000 organisms per ml. or more. In no quarter was there a cellular response as shown by the total cell count at the first sampling, but a marked reaction was noted in all nine quarters at the third sampling. In all quarters swelling of the gland occurred and lasted for three to eight milkings; the secretion was abnormal for six to ten milkings. The same authors(48) have used the Hadley-Wisconsin swab technique, which consists of pushing an infected swab 2–3 mm. into the teat canal. Swab-exposures with *Str. agalactiae* resulted in seven of seventeen quarters becoming infected. Organisms were recovered from all seventeen quarters for two or three milkings, but penetration of the canal was not considered to have occurred unless large numbers were found and there were clinical signs of mastitis. None of the quarters showed such severe clinical mastitis as was seen with the pipette method of infection, and the authors consider that the pipette method does not reproduce natural infection. Another difference noted between the two methods of exposure was that the pipette method did not infect two quarters which were already infected with other organisms, whereas the swab method infected five such quarters. Mundane(49) reports that the dipping of teat-cups in sodium hypochlorite solution considerably reduced the spread of infection by *Str. agalactiae*. He used two groups of cows. In one group the teat-cups were dipped in milk from an infected donor cow before milking each third cow. In the other group the procedure was the same but the teat-cups were dipped in hypochlorite solution after milking each cow. After 2 weeks twenty-five out of thirty-six quarters in the first group became infected, whereas in the second group only two quarters out of thirty-six became infected. It would be interesting to know what the result would have been if water had been used instead of hypochlorite solution. A series of experiments on methods to control the spread of *Str. agalactiae* in a dairy herd has been made by Hughes(50). Each experiment entailed the use of an infected donor cow which was always milked first, and it was considered that infection had been spread if the organism was found in the milk or on the teats, using enrichment methods. Spread of the organism was controlled when separate sterile udder cloths were used for each cow and at the same time hypochlorite was added to the washing water, and

two sets of teat-cups were used, one set being disinfected whilst the other was in use. When hypochlorite was omitted from the washing water all ten heifers in the experiment were infected sometime during a period of 21 weeks, and the milker's hands were regularly infected. When the hypochlorite was retained, but the same udder cloth used for all the udders, all ten heifers in the experiment were infected after 10 weeks, but the milker's hands remained uninfected. When, however, only the disinfection of teat-cups was omitted the infection spread to only six cows in 22 weeks or to four cows in 9 weeks (two experiments) and the milker's hands remained uninfected. Thus infection may be spread by using the same udder cloth for every cow, even though hypochlorite is used in the udder washing water, and by the hands if hypochlorite is omitted. Although infection was spread by teat-cups, the spread was not so rapid as by udder cloths or hands. Merilan & Herman(51) report that the morphological form of *Str. agalactiae* as found in the udder differs from that of many laboratory cultures. They claim that motility was exhibited by this organism, and in three instances cell multiplication was seen following a brief period of movement by pairs of diplococci. They found that the intramammary infusion of filtrates of short-chain forms, as found in mastitic milk, produced hardness and swelling of the infused quarter within 1–2 hr. and that this was followed by a rise in rectal temperature of 3–5° F. Abnormal milk persisted for 2–3 days. Subsequent daily infusions of the filtrate into the same quarter resulted in the continued production of abnormal milk but the swelling response decreased each time. The infusion of filtrate into another quarter of the same cow, however, caused responses similar to those of the initial infusion. Infusion of filtrates of long-chain *Str. agalactiae* produced only minor symptoms of irritation, although the milk at the first milking was abnormal.

D. *Mastitis caused by organisms other than* Streptococcus agalactiae

A severe mastitis problem associated with *Cryptococcus neoformans* is reported by Pounden, Amberson & Jaeger(52). Of 235 cows, 106 became infected and fifty-five developed visibly abnormal udders and milk. Treatment with antibiotics was found to be of no use, but reasonable success in control was obtained by segregation and improved sanitation. The origin of the infection could not be traced. The organism was identified by Emmons(53), who compared it with strains from human sources and found no difference. The pathology of this outbreak is described by Innes, Seibold & Arentzen(54). On macroscopic examination the affected udders were abnormally firm, fleshy and greyish white. The supra-mammary and deep inguinal lymph nodes were involved, and were from 2 to 10 times the normal size; they were waterlogged and had scattered haemorrhages. Microscopic examination of affected udder tissue showed lesions varying from pyogenic granulomata containing cryptococci, to scattered areas where the involved tissue was diffusely infiltrated with foamy histiocytes. In one animal the lungs were also affected. Simon, Nichols & Morse(55) also describe an outbreak of mastitis associated with *C. neoformans* in which fifty of 280 lactating animals were affected. Most of the animals became infected 4–8 weeks after parturition, and many of them had been treated with penicillin at the close of the previous milking period. Antibiotic treatment was not effective. Steele-Bodger(56) reports seventeen cases of mastitis in lactating cows associated with yeasts, seven being mixed infections with *Str. agalactiae* or staphylococci. In the mixed infections antibiotics or sulphonamides cleared the bacteria but the quarters remained hot and painful. In the ten quarters infected with yeasts there was rapid and marked induration followed by a gradual drop in milk yield. In all cases brewers' grain formed part of the ration. The writer claims that intramammary iodine was successful in the treatment of all but one quarter. Giving case reports of yeast infections of the bovine udder, Tucker(57) states that in no case was 'acute death' seen, and the majority of infections were self-limiting.

The incidence of chronic staphylococcal mastitis having the appearance of actinomycosis appears to be increasing in Sweden, according to Tanner(58) who has made a study of 144 udders which to the naked-eye had actinomycotic lesions. Bacteriological studies were made on ninety-eight cases; *Staph. pyogenes* was isolated from 84 of them, *Pseudomonas aeruginosa* from two, and mixed flora from the remainder. Microscopic examination of udder tissue showed granu-

lation tissue around colonies of gram positive cocci which were surrounded by radiating club formations, or gram-negative colonies in the two cases associated with *P. aeruginosa*. It is quite probable that such lesions may be responsible for some of the cases of so-called chronic staphylococcal mastitis which are resistent to antibiotic treatment.

In 1951, Stuart & Harvey(59) in Britain reported a type of bovine mastitis which resembled tuberculosis and which was caused by *Mycobacterium lacticola* and other rapidly growing acid-fast bacteria. More recently, Tucker(60) has given the first report of this type of mastitis in America. He describes an outbreak in a herd involving thirty-two out of 101 cows. He confirmed the experimental findings of Stuart & Harvey that the condition could be reproduced by the infusion into normal udders of oily, but not watery, suspensions of the organisms. Tucker(61) also reports a case of clinical mastitis associated with *Pasteurella multocida*. An outbreak of mastitis due to infection with an organism of Friedlander (Klebsiella) group is described by Buntain & Field(62). When first detected five cows were affected, but 7 months later twenty-eight of the thirty-eight cows in the herd were excreting the organisms in the milk. The onset of the condition was insidious with the presence of only a little mucoid material in the fore-milk, but later the milk became grossly abnormal. The authors consider that if aureomycin had been available in sufficient quantities the infection could have been eradicated. Eieland(63) reports two instances of mastitis caused by pneumococci type 15 and type 19 respectively. Penicillin promptly relieved the condition and the organisms rapidly disappeared. Group Ca streptococci infecting the udders of 220 cows on fifteen farms has been reported by Rømer(64). Treatment with penicillin was successful.

E. *General*

Work on the inhibition of micro-organisms by raw milk has been done by Wilson & Rosenblum(65) and Auclair & Hirsch(66). Wilson & Rosenblum report that lactenin, the antistreptococcal substance in raw milk, inhibits *in vitro* the growth of all group A streptococci, but in the cow's udder lactenin is inactive because the milk is at a low oxidation reduction potential and the organism can therefore become established. Streptococci of groups B, C, D and F, although they may be temporarily inhibited *in vitro* ultimately achieve full growth. Auclair & Hirsch confirmed the existence of two inhibiting substances in raw milk, lactenin 1, occurring mainly in colostrum and lactenin 2, occurring mainly in milk. They found that milk and especially colostrum possess stimulating properties which enable *Str. agalactiae* to overcome inhibition. After making studies of the growth of a strain of *Str. agalactiae* in milk from infected and uninfected cows, Murphy & Stuart(67) reached the conclusion that the inhibition of growth by normal, uninfected milk would seldom, if ever, represent a significant barrier to infection of the gland. The possible influence of the ration fed to cows on the activity of *Str. agalactiae* in the milk has been studied by Pounden, Hibbs & Edgington(68) who used acid production to estimate the growth of the organism. Milk from cows fed on better quality roughage and small quantities of grain was apparently more resistant to growth of the organism than that from cows fed on lower quality roughage and larger quantities of grain. Using the same method, Pounden(69) found that the resistance of milk to the growth of *Str. agalactiae* lessened as lactation progressed.

Studies on milking technique have been made by Mochrie, Hale, Eaton, Elliott, Plastridge & Beall(70). Twelve first-calf heifers which were free from mastitis at the fourth week of lactation were milked at three different levels of vacuum. No difference in leucocyte count, chloride content, pH values or freedom from mastitis was found as a result of the different pressures. Similar results were obtained by Mochrie, Hale, Eaton, Johnson & Plastridge(71) using twelve cows, but in addition they found that increase in milking time resulted in an increase in the leucocyte counts and chloride values, and a drop in the milk yield. Helmboldt, Mochrie, Plastridge, Eaton, Easterbrooks & Hale(72) made histopathological examination of eleven heifers milked at different pressures and for different times, and concluded that the lesions present had no correlation with the milking technique.

Analysing the milk yield and incidence of infection with *Str. agalactiae* in four herds over a period of 9–13 years, McLeod & Wilson(73) found that fifty-seven cows which became infected in the second to the sixth lactations, yielded more milk before infection than did uninfected animals in corresponding periods, and that in thirty-two cows infected in the second to fifth lactations, there was an apparent loss of 24% of the yield. Crossman, Dodd, Lee & Neave(74) have recorded the individual quarter yields of cows using an individual quarter milking bucket designed by Hall(75). They found that subclinical infections of the udder with streptococci or haemolytic staphylococci may persist for months and may either have no measurable effect or may cause a gradual reduction in milk yield of the affected quarter and reduce the percentage of solids-not-fat in the milk. They observed that both results may occur in the same udder but in different quarters. Following penicillin treatment of clinical cases of mastitis, they found little recovery in yield until the next lactation, when recovery might be complete. Dodd & Neave(76), who point out that slower milking cows tend to be culled at an earlier age than fast milking cows, found that in the first lactation there was a marked and progressive increase in the amount of mastitis, caused mainly by coagulase positive staphylococci, with the increasing speed at which the cows milk.

From the data obtained from eleven North Carolina dairy herds over a period of 5 years, Legates & Grinnells(77) made an estimate of heritability of resistance to mastitis of $0{\cdot}27 \pm 0{\cdot}10$.

For the evaluation of teat erosion, Pirozok, Mochrie & Helmboldt(78) describe a method for making casts of the exterior of bovine teats for comparison with casts of the same teats made later in life.

II. CONTAGIOUS ABORTION

A. *Diagnosis*

Considerable attention has been paid in the past few years to diagnostic tests for contagious abortion using milk and stained antigen. A milk plate test, which requires the same equipment as the blood-serum plate test, has been described by Blake, Manthei & Goode(79). The milk to be tested is diluted with negative milk and 0·08 ml. placed on a plate; 0·03 ml. of ring test antigen is mixed with it and the result read after 12 min. at room temperature. The authors consider that this test overcomes the drawbacks of the milk ring test in that it can be used with goat's milk, milk from cows in early and late lactation, homogenized milk, sour milk, skim milk, mastitic milk and cream. Gregory(80) describes a rapid milk agglutination test which is done in tubes. He gives the method of preparing the antigen, stained with reduced tetrazolium salts, and of preparing a diluting fluid from milk. Skim milk is used for the test and therefore the coloured agglutinated organisms do not rise with the cream as in whole milk, but form a deposit at the bottom of the tube. He gives four degrees of agglutination: (1) less than 25% of the bacteria in the sediment and colour left in the fluid above; (2) 25–50% of the bacteria in the sediment; (3) the majority of the bacteria in the sediment, but some colour left in the supernatant fluid; (4) complete agglutination, with milk-white supernatant fluid. The test can be used for a quantitative estimate of the specific antibody in milk, and also as an aid to diagnosis in herds or in individual cows.

Ferguson & Robertson(81) have published a report on 438 simultaneous blood serum and milk ring tests. The ring test was 93% correct in detecting cows whose blood serum was positive to the agglutination test. Using the plate serum agglutination test as a standard for evaluation, Morse, Pope & Grota(82) made a comparison of the milk ring test and the capillary tube test. The milk ring test agreed with the blood serum test in some 76% of the comparisons made, whereas the capillary tube test agreed in only 72%. They found a lower percentage of false positives and false negatives with the milk ring test than with the capillary tube test. Morse, Smith & Schmidt(83), however, compared these two tests with the milk whey and blood serum agglutination tests on 243 samples from sixty-three cows with titres from 1:25 to above 1:400, and concluded that the capillary tube test appeared more sensitive than the milk ring test and was in closer agreement with the whey agglutination test. They do not consider, however, that either

test can be recommended as an unqualified substitute for the whey or serum agglutination tests. Fedyushin & Alexeyeva(84) compared the milk ring test with the rapid plate and the tube agglutination tests and the complement-fixation test on 403 cows from one control and three infected herds. The milk ring test gave the largest number of positive reactors in each herd. There was fairly close agreement between the three serological tests, which indicated about half as many reactors as the milk ring test. Driver & Roepke(85) consider that three milk ring tests will serve to locate 85% of newly infected herds and 95% of new reactors. Any weakness of the test as regards accuracy is more than offset by the frequency with which it can be applied in a control plan. Mingle(86) reports that in the co-operative brucellosis control and eradication programme in the U.S.A. 670,532 herds (12,000,000 cattle) have been screened by the milk ring test, and 26·2% were classified as 'suspicious'. The Joint FAO/WHO Expert Committee on Brucellosis(87) is of the opinion that although the milk ring test is referred to in many countries as the ABR test (Abortus Bang Ringprobe), it should in future be referred to as the milk ring test, since it is used for the milk of animals infected not only with *Br. abortus* (Abortus Bang) but with any one of the species of Brucella, and it is specially used for milk.

Comparison of a haemotoxylin stained antigen with several preparations made with tetrazolium have been made by Rislakki & Stenberg(88). All were equally specific, but the tetrazolium stained antigens were more sensitive and also easier to prepare and less expensive than the haemotoxylin stained antigen. Bendtsen(89) describes the preparation and use of a milk ring test antigen stained with tetrazolium salts. Martelmans & Vercruysse(90) advocate the use of merthiolate (sodium-ethyl-mercuri-thio-salicylate) for increasing the keeping properties of cattle sera collected in the field and submitted to the laboratory for test.

The anamnestic reaction has been used by Barner(91) to differentiate vaccinal from infection titres of *Br. abortus* in cows. He found that no elevation of titre resulted from the vaccination of infected animals whether they had been previously vaccinated or not, and that with a few explainable exceptions all revaccinated non-infected animals showed a marked rise and subsequent decline in titre. This reaction in the non-infected animals is regarded as anamnesia or 'recollection' of the first vaccination. Barner, Oberst & Atkeson(92) obtained similar results using dead strain 19 vaccine.

Hess(93) has described studies made on a non-specific brucella-agglutinating substance in bovine serum, which can be differentiated from the specific brucella agglutinin in that it will agglutinate various other organisms. The differences between the two substances are shown in Table 1.

Table 1. *Methods of distinguishing between the specific and non-specific brucella agglutinating substances*(93)

Basis for the differentiation of the agglutinating substances	Non-specific substance	Specific substance
Removal from serum by absorption with bacteria	Accomplished with a variety of organisms	Accomplished with brucella only
Heat required for inactivation	70° C. for 10 min.	90° C. for 10 min.
Chromatographic adsorption of cellulose	Adsorbed	No adsorption
Yield obtained by elution from *Br. abortus* with distilled water	Up to 38%	Less than 2%
Concentration of ammonium sulphate for precipitation	30% saturation	50% saturation
Relationship to γ-globulin	No association	Contained in the γ-globulin fraction

By means of cross-agglutination reactions, Morse, Ristic, Robertstad & Schneider(94) have determined that *Salmonella pullorum*, *Vibrio foetus* and brucella species are antigenically related. Pasteurella and *V. comma* share antigenic compounds with brucella species but not with *V. foetus*. Renoux(95) discusses the blocking antibodies which may be present in the serum of animals infected

with brucellosis. These blocking antibodies may cause negative agglutinations in tubes of one or more dilutions, whereas the dilutions above and below this 'zone' may show agglutination.

Petrov & Sidorova(96) comparing the complement-fixation test with the tube agglutination test, claim that the former gave about twice as many reactors as the latter. Rice, Boulanger, Mackie & Moore(97) used a conglutination complement-fixation test in association with the plate and tube agglutination tests and a haemolytic complement-fixation test. On 2191 samples of blood taken over a period of 2 years from 174 non-vaccinated animals in a herd in which an outbreak of brucellosis had occurred, the conglutination complement-fixation test and the plate test were the earliest to detect low degrees of reaction. The haemolytic complement-fixation test was the least sensitive. Rossi & Dutillory(98) have described a test to demonstrate the presence of bacteriotrophins in bovine milk and blood. Loktyeva(99) has suggested an eye test for the diagnosis of brucellosis in cattle but has not given the method of preparing the antigen. He claims that more animals were isolated in 15 days with this test than in 4 months with the agglutination and complement-fixation tests.

B. *Bacteriology*

Cameron & Meyer(100) have tested the effect of the three major synthetic dye groups (quinone-imide, phenyl methane and xanthine) upon the growth of the three species of Brucella. The only consistent pattern found within a group was the ability of the xanthine compounds to inhibit the growth of *Br. melitensis* at concentrations much lower than those permitting growth of the other two species. They concluded that the structure of the dye *per se* was not wholly responsible for the variation in growth. The Joint FAO/WHO Expert Committee on Brucellosis(87) has stressed that in the isolation of brucella, cultures should be kept for 35 days before being discarded, and that in identification of the three species, dissociated strains may give misleading results. Pickett, Nelson & Liberman(101) recommend that the colony selected for differentiation should appear smooth when examined under obliquely transmitted light and that the stability of the organisms in acriflavine should be examined. In this way 258 strains from man and animals were screened and twenty-six of them were rejected. The remaining 232 strains were tested for urease activity, sensitivity to thionin, basic fuchsin, crystal violet and pyronin, for sulphide production, and sensitivity to diethyldithiocarbamate and to azur A and saffron O. Of these 232 strains 214 fell into three well-defined groups corresponding with the three generally recognized species of Brucella.

Renoux & Carrère(102) criticize Huddleson's methods of differentiating the three species of Brucella, and claim to have changed *Br. abortus* into *Br. melitensis*, as judged by his criteria, by serial passage. Cameron & Meyer(103) passaged a smooth culture of strain 19 *Br. abortus* through twenty-one swine, over a total period of 406 days, and it showed no evidence of variation. The information suggests that any of the three species may become parasitic in a susceptible host without modifying the cultural characteristics. Renoux(104) has suggested that the three species of Brucella should be named according to the suggestion originally put forward by Meyer & Shaw(105), i.e. *Br. brucei* var. *melitensis*, var. *abortus* and var. *suis* (American type). The Danish type would be named var. *thomseni*. Renoux(106) describes a method of differentiating the three species by means of an 11·0 mm. filter disk dipped in a 1% solution of diethyldithiocarbamate and placed in the middle of an agar plate already sown with the organism. *Br. suis* shows a 20–30 mm. zone of inhibition around the disk, and *Br. abortus* a zone of weak growth around the disk then a brown ring, a white ring and a 1·0 mm. zone of inhibition. *Br. melitensis* shows a more or less intense growth around the disk surrounded by a clear opaque blue ring, around which is a 3–4 mm. zone of inhibition. Renoux has used the method to differentiate thirty-one typical and eleven atypical cultures of Brucella. Huddleson, Richardson, Warner & Baltzer(107) have given a method of differentiating the three species by means of tetrazolium salts.

Further work has been done on the toxicity of amino-acids for Brucella. Elemental sulphur has been isolated by Schuhardt, Rode, Oglesby & Langford(108) from autoclaved solutions of

cystine. They found that colloidal dispersions of this and other sulphur samples were toxic for certain strains of Brucella in concentrations as low as 0·06 mg/ml. of culture medium, and that by all of several tests the toxicity appeared to be the same as the anti-brucella toxicity of autoclaved cystine or toxic tryptose (see previous review(24)). Yaw & Kakavas(109) have studied the effects of D-amino acids on *Br. abortus*. The growth of three strains of this organism in broth was inhibited by added D-amino acids at levels at which the L-forms did not exhibit such an effect. D-phenylalanine and D-methionine caused the greatest amount of inhibition of growth. The DL-forms of all the essential amino-acids had an inhibiting effect, DL-lysine being the least inhibiting. The D-amino acids appear, therefore, to have a bactericidal effect on *Br. abortus* as well as a bacteriostatic effect. Cameron & Meyer(110) report that the thionine inhibition to the growth of *Br. suis* has been counteracted by the addition of glutamic acid. Glutamic acid failed to increase the tolerance of the other two species to thionine. Rapid population changes, involving the establishment of non-smooth mutants of the three species of Brucella are reported by Braun, Kraft, Mead & Goodlow(111). These changes occurred when the organisms were transferred serially in broth containing increasing concentrations of penicillin. This effect appeared to be associated with increased accumulation of alanine.

C. *Vaccination*

The comparison between different routes of vaccination of animals with *Br. abortus* strain 19 vaccine has been made by various workers. Gregory(112,113,114,115) in a series of four papers describes trials in which heifers were vaccinated when 7–10 months old, with either 1·0 ml. intracaudally or 5·0 ml. subcutaneously. These animals, twenty in each group, were exposed, with a group of twenty unvaccinated controls, to a heavily infected environment. In the control group 95% became infected and 52% aborted; in the intracaudal group 40% became infected and 29% aborted; in the subcutaneous group 35% became infected and 14% aborted. Two groups of twenty-two cows each, vaccinated by both methods, were exposed a second time during their second pregnancy. In the intracaudal group 18% became infected and none aborted, and in the subcutaneous group 9% became infected and none aborted. Gregory concluded that intracaudal vaccination with strain 19 confers protection equal to that afforded by subcutaneous vaccination. Manthei, Mingle & Carter(116) have compared immunity and agglutinin response in cattle vaccinated with strain 19 by the intradermal and subcutaneous routes. They found no difference in immunity and agglutinin response in cattle inoculated as yearlings with 5 ml. of vaccine subcutaneously or with 0·2 ml. intradermally. The agglutination titres obtained were above the diagnostic level in a high percentage of the animals, and most of them were not resistant to the excessive conjunctival exposure dose of virulent *Br. abortus* during the first pregnancy. Manthei(117) concludes from the above data that further research is needed concerning methods of vaccination before recommendations can be made regarding the minimum dose and the most effective method of vaccination. Cotton(118) has made a study of the post-vaccination responses in groups of calves vaccinated intracutaneously and subcutaneously with strain 19 vaccine. Eight calves, which had high opsonocytophagic reactions prior to vaccination intracutaneously, failed to develop high agglutination titres. Another eight calves, also with high opsonocytophagic reactions, were vaccinated subcutaneously, and five of them did not develop titres. The author believes that sufficient immunity develops in calves suckling their dams, as indicated by the high opsonocytophagic reactions, to reduce the efficiency of calfhood vaccination. Sieiro(119) has compared the immunity to brucellosis in cattle by strain 19 vaccination by intradermal and subcutaneous routes. He found that, against a test dose of 5 million organisms, 100% of the animals vaccinated intradermally were protected, whereas only 50% of the animals vaccinated subcutaneously were protected. Against a test dose of 10 times as many organisms the figures were 75 and 11% respectively.

Bosgra(120) discusses the advantages of using strain 19 vaccines dried from the frozen state. He describes the technique used in cultivating a high yield of living strain 19 organisms and of drying the suspensions in a dextrose solution from the frozen state, with a survival rate of about

50% of the organisms. Another method for the production of a desiccated strain 19 vaccine has been described by Thorne (121). The vaccine is desiccated in a skim milk-phosphate buffer medium with added lactose. The loss of viability during desiccation was less than 50%, and little further loss was found on storage for 6 months. When a vaccine is being transported over long distances, it may be seriously affected by agitation, and in the tropics by temperature. The use of a desiccated vaccine is helpful in overcoming these difficulties. In Thorne's procedure reconstituted vaccine, at a dose of 1·0 ml., is administered into the caudal fold. This route appears to give as good results as the subcutaneous or intracaudal methods.

Berman, Beach & Irwin (122) vaccinated three groups of heifers with strain 19 at the age of 8 months. Two of these groups were revaccinated at 14 and 20 months respectively. In the third pregnancy all three groups were exposed by the conjunctival route to 12×10^6 organisms of *Br. abortus* strain 2308, and no increment of resistance, engendered by revaccination was apparent. Sieiro & Rosenbusch (123) obtained superior anti-infectious values, in contrast to anti-abortion values, by vaccinating with a non-agglutinogenic strain of *Br. abortus* than were obtained with an agglutinogenic strain. Washko & Hutchings (124) found that vaccination with strain 19 did not protect two heifers against infection with *Br. suis* injected into the udder 3 months after calving.

Comparative tests on the protective value of Huddleson's mucoid vaccine and strain 19 vaccines have been reported. Edgington, King & Frank (125) selected twenty-one heifers from a brucella-agglutination negative herd, and, at the age of 8 to 15 months, they either vaccinated them with Huddleson's 'M' vaccine or strain 19 vaccine or they left them unvaccinated. When 4–6 months pregnant each animal was submitted to conjunctival exposure of 750,000 viable organisms. The protective value of the vaccines was assessed on the basis of recovery of *Br. abortus* by cultural methods from each heifer or aborted foetus at or near the time of calving. The organism was recovered from 83·3% of the unvaccinated controls, from 50% of the 'M' vaccinated and from 20% of the strain 19 vaccinated animals. Just after vaccination the 'M' vaccinated animals were negative to the agglutination test, whereas the strain 19 vaccinated animals gave high titres. The values of the 'M' vaccine may lie in the absence of agglutination titres in vaccinated animals. Edgington & King (126) report similar results for a total of sixty-eight heifers, and came to the conclusion that neither vaccine gave complete protection, but that strain 19 gave greater protection than the 'M' vaccine. After trials on 228 heifers between the ages of 4 and 8 months, Bryan (127) concluded that the 'M' vaccine failed to produce a significant degree of resistance against brucellosis, whereas strain 19 vaccine did induce a significant degree of resistance of a relative type. Schoenaers (128) vaccinated twelve animals subcutaneously with 'M' vaccine, twelve intravenously and left twelve unvaccinated as controls. He found 20 months later that the vaccinated cattle were no more resistant to infection by conjunctival instillation of *Br. abortus* cultures than the controls.

Though tests with Huddleson's 'M' vaccine are discouraging, the same work tends to show that the results from strain 19 vaccination are themselves not very satisfactory. It is well to note, therefore, that work is being done on other methods of vaccination. Schlingman & Manning (129) prepared a vaccine from a virulent culture of *Br. abortus* by sterilization with ultra-violet light and precipitation with alum. It was tested on twenty-eight cattle, which were later exposed to homogeneous and heterogeneous strains of *Br. abortus*. On serological and bacteriological grounds the vaccine was considered to have conferred considerable immunity. Different brucella antigens have been compared by Live & Guiliani (130) who have studied the protective effect of sera from the vaccinated animals upon brucella infections in mice. Sera from cattle which had been injected with ether-killed *Br. abortus* combined with 'Falba' and mineral oil had a much higher protective action against lethal *Br. abortus* infection in mice than sera from cattle injected with live strain 19 vaccine and having similar agglutination titres. Similar results were obtained with sera from vaccinated guinea-pigs. Sera from guinea-pigs infected with virulent *Br. abortus* had variable protective activity for mice. Gwatkin & Dzenis (131) report that subcutaneous injection of heavy suspensions of ether-killed *Br. abortus* in saline and oil emulsion

gave some protection to guinea-pigs against a light exposure by eye which infected the control animals. The control animals showed lesions in the spleen and *Br. abortus* was isolated from the tissues. The same authors(132) have compared the immunity produced in guinea-pigs by strain 19 in saline and in a saline and oil emulsion. At 12 months five out of seven of those vaccinated with the saline and oil emulsion met the requirements of resistance after challenge, whereas only one out of seven of those vaccinated with the saline vaccine and none of the controls met these requirements. Further, the same authors(133) have found that the addition of hyaluronidase to heat-killed microaerophilic virulent *Br. abortus*, given by the subcutaneous, intradermal, intraperitoneal or intraocular routes, had no observable effect on the antigenicity of the organisms as shown by agglutinin production or resistance to infection.

D. *General*

During the period under review considerable attention has been paid to the pathology and pathogenesis of brucellosis. Braude(134) has compared the pathogenicity of the three species of Brucella for guinea-pigs. *Br. suis* produced the most numerous and destructive lesions, but in general the condition of the animals remained fairly good. *Br. abortus* showed evidence of tissue involvement with the development of small non-suppurative granulomata and marked enlargement of the spleen, but the animals remained in good health and gained in weight. *Br. melitensis* was intermediate in its destructive capacity but was the most debilitating. It appears that the good defence of guinea-pigs against *Br. abortus* is associated with the presence of non-suppurative lesions and marked enlargement of the spleen. Godglueck(135) also found that the lesions caused by *Br. suis* were larger than those produced by the other two species, and were frankly exudative, formed abscesses and contained many giant cells. The infectious granulomata caused by *Br. melitensis* also contained giant cells but in small numbers. Braude(136) puts forward evidence that, in experimental brucellosis, the development of granulomata does not appear to depend on the hypersensitivity of the animal. Studying superinfection in experimental brucellosis, Pollack, Kelly, Gorelick, Braun & Victor(137) found that guinea-pigs with *Br. abortus* infection of 2 weeks or longer duration, were partially resistant to superinfection with *Br. suis* in that the lesions were less intense and often culturally negative for *Br. suis*. It did not appear to be necessary for *Br. abortus* to be still present in the animal. Kelly, Gorelick, Silverman & Braun(138) found that this partial resistance became evident within 5–10 days of the original infection. When, however, both species were inoculated at the same time, the recovery of *Br. suis* was similar to that from the controls, which had been inoculated with this organism alone. The same authors(139) found that *Br. suis* infection could be readily imposed on a *Br. melitensis* infection at 5 or 15 days, but less easily on a homologous infection with another strain of *Br. suis*. *Br. melitensis* could be imposed on a *Br. suis* or on another strain of *Br. melitensis* at 5 days, but was inhibited to some extent at 15 days. *Br. abortus* was largely prevented from infecting when imposed on a 5- or 15-day infection with *Br. suis*, but the authors suggest that since *Br. abortus* is slow in development, an interval to sacrifice of longer than 4 weeks might have altered this result. Pomales-Lebrón & Fernández(140) used an anaerobic culture of *Br. abortus* for primary inoculations of guinea-pigs and an aerobic strain for reinoculation. In only one instance did the organism of reinoculation persist in the tissues in the presence of that of the primary inoculation. Victor & Valliant(141) found that the opsonin titre of guinea-pigs is related to the extent and intensity of the specific lesions in the animal, and conclude that brucella opsonins are formed in the specific lesions. Victor, Pollack, Raymond & Valliant(142) described a modified method of titrating opsonin using heparin as an anticoagulant.

Washko, Donham & Heimlich(143) succeeded in infecting four female calves by conjunctival exposure to *Br. abortus* after injecting gonad-stimulating hormones. Four control calves, which were not injected with hormones, failed to become infected as measured by the blood serum agglutinin response. The infected animals, when exposed to virulent *Br. abortus* during their first pregnancy, failed to show increased resistance as a result of prepubertal infection, though a possible alteration in the abortion rate was observed. Washko, Donham, Hutchings & Heim-

lich(144), using *Br. abortus*, infected fifteen cows by way of the conjunctival and three by the intrammamary route. After slaughter of the animals they made cultures from many of the organs. The organism was recovered from widespread areas in the carcasses of nine of the eighteen cattle. In all cases of isolation from the udder, the organisms were also recovered from tissues other than mammary gland or reproductive organs. Though there is no conclusive evidence that the bull is capable of transmitting brucellosis by natural service, Manthei(145) has succeeded in infecting eight of twelve cows by interuterine insemination with infected semen. Estimating the concentrations of iron, copper, zinc and cobalt in the blood and livers of cattle, Gessert, Berman, Kastelic, Bentley & Phillips(146) found that cattle which contracted brucellosis had approximately the same concentrations of these minerals in their livers as cattle which were similarly exposed without becoming infected.

With regard to treatment of brucellosis, King, Venzke & Edgington(147) found that blood plasma, sulphadiazine, streptomycin, colloidal manganese and aureomycin, singly or in combination, when administered to sixteen cows caused only a temporary cessation in the shedding of organisms in the milk. Kelly & Gorelick(148) report that the combined use of streptomycin, sulphadizine and aureomycin proved the best of several treatments in guinea-pigs infected with *Br. suis*. Comparing terramycin, dihydrostreptomycin and sulphadiazine against brucellosis in the white rat, Jacotot & Vallée(149) found that terramycin was the most efficient, and sulphadiazine the least efficient. The three drugs used together always brought about recovery. Immune serum given with an antibiotic in a great number of cases hindered the action of the antibiotic.

Referring to brucellosis in man the Joint FAO/WHO Expert Committee on Brucellosis(87) stated 'the basis of prevention of human brucellosis lies in the control and eradication of the disease in animals'. It draws attention to the increasing frequency of cross-infections with any of the three species in various domestic animals. Vaccination of pregnant animals with strain 19 vaccine may cause the excretion of the organism for a period of a week following parturition. Ferguson & Robertson(81) found that 53% of the milk coming into Edinburgh was positive to the milk ring test, but point out that strain 19 vaccination may have been responsible for this. Dalrymple-Champneys(150) states that strain 19 vaccine has been shown to be safe from a public health point of view. No case is known of a person contracting undulant fever from the milk of vaccinated cattle, but the disease has occurred occasionally after accidental injection of the vaccine. He points out that Britain is fortunate in that there is in the country at present only *Br. abortus* and an aberrent melitensis-like organism. The latter organism does not cause undulant fever.

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REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION A. PHYSIOLOGY OF DAIRY CATTLE

PART II. PHYSIOLOGY AND BIOCHEMISTRY OF RUMINATION

Since the time of the previous review in this series by the present author it has been increasingly realized that the ruminant's adaptation to its herbivorous diet is very far-reaching. The biochemical consequences of its need for coarse roughage are still being worked out and have necessitated a reassessment of the causes of bovine ketosis, of the function of lower fatty acids in its blood and of the reasons for its large requirement of cobalt. Recent work on all these three aspects of ruminant metabolism is discussed in this review. The cow's chief function is to convert into milk vegetable materials, many of which are unsuitable for human consumption. Accordingly, some of the nutritional aspects of this conversion process have been discussed in the review. The literature on the subject has been reasonably well listed, but the author cannot claim that he has not perhaps inadvertently omitted certain pertinent new work.

GENERAL

Sherwood & Jones (1) found that alfalfa hay plus a mixture of wheat, barley and oats gave 20% more milk than the hay alone. Milk yield was also enhanced by adding the succulents squash and Jerusalem artichoke to the hay diet. Groups of cows receiving the concentrates needed 5–6 lb. more total digestible nutrients to produce 100 lb. milk, and the authors conclude that feeding of succulents is profitable only if they produce nutrients as cheaply as does hay. Watson, Davidson, Kennedy & Sylvestre (2) compared the digestibility of rations of hay plus increasing amounts of oats. As oats increased the digestibility decreased. This effect was most marked with barley, not so marked with oats and was only slight with linseed oil meal. Feeding standards are still the subject of informed criticism in Europe (3,4,5). Nehring (3) concludes that Kellner's starch equivalents cannot be used for pigs without prior respiration calorimetry. Blaxter (4) finds large discrepancies between different methods of computing rations for livestock, and Nicholls (5) enters a plea for food recording as a complement to milk recording. The trouble with all attempts to define standards for grazing animals is the extreme variability from year to year, from field to field, from treatment to treatment and from animal to animal of the nutrients provided by grass and hay or silage. Much current research all over the world is designed to measure more accurately the nutrient intake of cattle and sheep from pasture, with a view to striking a better balance between protein and fibre in the diet, always remembering that the content of fibre biologically best for maximal production per cow may not necessarily give the maximal production per acre. This may come from more cows per acre at somewhat more fibre per cow than would give maximal production per cow. In this connexion the observations of Hancock (6) are pertinent. He points out that the maximum production per animal does not necessarily yield the greatest net returns. In practice this can be achieved by a number of cows per acre too high for maximum productivity per cow or for perfect health and comfort. Under such conditions grazing time is long and rumination time short.

Sikka (7), in a study of some 2000 lactations, has shown positive correlations of + 0·70 and +0·37, respectively, between total yield on the one hand and maximum yield and persistency on the other. There was little correlation between maximum yield and persistency ($r = -0{\cdot}17$). Rendel & Robertson (8) estimate the present productive life of a cow as about four lactations, and they say that an increase to five would produce only 1% more milk. They state that longevity is of minor importance compared with the yield of milk per herd. The main advantage of increased longevity would be an increase of cross-bred beef stores. On the average only one in

every three calvings produces a heifer which reaches lactation. Discounting dairy cows mated to beef bulls, therefore, the maximum replacement is less than one-third so that more than three lactations are necessary for equilibrium of herd numbers. The annual yield of milk (9) is poorly correlated with live weight. Milk yield is greater in cows of greater blood pressure (10), as indeed the American workers showed earlier.

GRAZING BEHAVIOUR AND DIETARY HABITS OF RUMINANTS

This subject has been reviewed by Tribe (11,12) who, with others, has also contributed new observations of his own (13,14,15). Pregnant ewes were divided into two groups (13), one receiving a supplement, the other not. While allowed to graze a separate field the supplemented group spent a shorter time grazing. When, however, the groups were put in the same field their grazing times were equal. Ewes of the supplemented group, carrying twins, grazed 1 hr. longer than those with singletons. This latter difference was not found in unsupplemented ewes with twin and single pregnancies. When presented with a choice of a protein concentrate, carbohydrate, hay, minerals and water, eight individually housed ewes failed to choose a balanced diet, and three of them developed a condition resembling pregnancy toxaemia. Gordon & Tribe (15) have shown the importance to sheep of frequent feeding. Castle, Foot & Halley (16) found that when on better pastures dairy cattle grazed less, walked less and took fewer drinks. Burton & Castle (17) have described the construction of a sort of J. L. Baird Noctovisor for observing cattle in the field at night by infra-red radiation. Hughes & Reid (18), at the British Grassland Research Station, found that both cattle and sheep grazed longer in early morning and late afternoon than at other times. In winter grazing went on for twice as long as rumination. In winter there was a marked midnight grazing period for sheep, and in autumn a similar phenomenon was noted with cattle. Probably owing to change of the herbage as the season advanced, the time cattle ruminated was only slightly less than the grazing time in midsummer. In spring and autumn the time spent in grazing was twice as long as the time spent in rumination. Habits varied considerably from one animal to another. In these English studies (18) (latitude 52° N.), and in similar ones by Hancock (6) in New Zealand (latitude 37° S.), grazing occurred mainly during the day. In Fiji (latitude 18° S.), Payne (19) found that cattle did 67% of their grazing at night, and that when hot weather diminished grazing, the day grazing suffered the greater diminution. Studies of the effect of management on productivity and grazing behaviour have been made at this Institute (20, 21, 22) (latitude 55° N.). Waite, Macdonald & Holmes (20) found cows spent 9 hr. grazing as against $6\frac{1}{2}$ hr. recorded by Castle *et al.* (16). This time difference may have been due to the fact that the cows in the latter observations, but not in the former, received supplementary food (13 lb. concentrates per cow per day). In the former experiments (20) half of the cows were close-folded by electric fences. This reduced the herbage intake, but did not affect the milk yields or grazing behaviour. Cessation of grazing was, as in experiments (18), determined by the setting of the sun. When the grass was more lush less time was spent grazing. There was no apparent correlation between the duration of grazing and either yield of milk or stage of lactation. This is in contrast to Tribe's observations on ewes carrying one and two lambs. Holmes, Waite, Fergusson & Campbell (21) found that close folding at a rate of fifty to eighty cows per acre or rotational grazing at a rate of six to eight cows per acre gave comparable yields of milk per cow. During 114 days, of which each group spent 57 days on each regime average yields per cow were 29·5 and 29·2 lb./day for close-folding and rotational grazing respectively. Close folding gave 550 gal. of milk per acre from a cocksfoot ley and 582 gal. from permanent pasture. Rotational grazing of the same pastures gave 450 and 351 gal./acre from cocksfoot and permanent pasture respectively. In these same experiments (22) dry-matter intakes were estimated to be 18–27 lb. for close-folding and 28 lb. for rotational grazing per cow per day. Estimates of intakes of SiO_2, Ca, P and proximate principles in the pasture eaten were made from chemical analyses throughout the experiments. The effect of reafforestation in decreasing the availability of forage for the Canadian moose has been studied (23). Its normal habitat appears to be the edge of the advancing forest. Walker (24) reports that bucket-fed calves will take much more milk than they would get by normal suckling.

Vasilets & Golushko(25) recommend cold rearing of calves in shelter rather than more adequate housing, and Maymone & Carusi(26) state that the sheltering of sheep during winter nights increases their milk yields.

In a comparison of rates of fattening, Callow(27) states that young animals fatten more slowly than old ones, and thus deposit relatively less fat and relatively more protein. Cows fatten more slowly than old steers and deposit nearly twice as much protein as do steers during fattening. From records of yields and of intakes of food from grazing and supplements, Sjollema(28) concludes that protein poor supplements such as rye-straw, potato flakes and dried sugar-beet pulp are of value, while the growth of grass is lush and plentiful, but are deleterious during drought. Rotational grazing of improved pastures is of increased value when supplements poor in protein are given. Klosterman, Bolin, Buchanan & Bolin(29) found that protein-poor rations caused the blood of pregnant ewes to become poorer in serum albumin and in oxyhaemoglobin, without any change in serum globulin. Williams, Sylvestre, Bowstead, Ewen, Myhr & Peters(30) reported that the maintenance of the weight of pregnant ewes and the birth weight and vigour of their lambs were better in ewes fed legume hay than in ewes fed non-legume hay. Garner(31) found that the globulin content of the blood of Nigerian Fulani cattle increased with age up to 5 years. Gorbacheva(32) found that the basic amino-acids were less well utilized for milk production by cows giving $2\frac{1}{2}$ gal. than by cows giving 4 gal. of milk. This is as would be expected on the theory advanced by the reviewer in the two previous reviews in this series. The cow with the lower production would be on a lower plane of nutrition and would thus allow a longer time of contact of incoming food with rumen bacteria, thus causing a loss of these amino-acids in the elaboration of less essential ones from them. Even in the rat(33) for maximum efficiency of amino-acid metabolism there is an optimum mixture of both essential and non-essential amino-acids. New amino-acids have been found α-aminoadipic in corn protein(34) γ-methylene-glutamic and γ-methylene glutamine in the peanut and diaminopimelic in bacteria(35). In brain γ-aminobutyric acid has been identified(36,37). The fixation of the carbon from intravenously administered $NaHCO_3$ has been studied by Kleiber and his colleagues(38,39). Carbonate carbon can be shown by using ^{14}C to be incorporated into lactose, into casein and into the fat of milk. Bouckaert, Oyaert & Peeters(40) found uptake of amino-acids from blood perfusing the isolated udder, while Campbell & Work(41) found that milk protein originates chiefly from blood amino-acids.

WATER

In a cold environment(42) cows ate 25% more food and maintained their milk yields. Water consumption did not change. Riek, Hardy, Lee & Carter(43) kept sheep at 30–45° C. for 7 hr./day once or thrice per week. Losses of water through respiration and excreta left 40% not accounted for. This was presumed to be true perspiration through epithelial glands. Herefords drank somewhat more water than Brahmans when in a warm environment, with drinking water at 88·2° F.(44). Mullick, Murty & Kehar(45) found that in India steers drank more water (15·1 kg./day) in summer than in winter (9·5 kg./day). The desert rat *Dipodomys merriami* is exceptional among mammals, since it can drink sea water and excrete the excess salt in its urine(46).

THE USE OF MARKERS

Schürch, Crampton, Haskell & Lloyd(47) found the chromium method suitable for pigs fed to appetite in the barn. Reid, Woolfolk, Hardison, Martin, Brundage & Kaufman(48), in studying their 'natural chromogen' method further, have found a correlation of $0{\cdot}995 \pm 0{\cdot}001$ between the measurement of the chromogen in the forage and the resulting faeces, so that the concentration of chromogen in the forage could be deduced from the faecal analyses. A comparison, using four different forages, of their method with the direct one gave $r = +0{\cdot}99 \pm 0{\cdot}004$. They devised the method as a means of estimating intakes of forage from faecal analyses. They state that use of chromium oxide simultaneously to estimate total daily faecal output would dispense with the use of faeces collection bags and make possible the simultaneous estimation of both intake and digestibility of the forage consumed by grazing cows. Kane, Jacobson & Moore(49) have re-

ported diurnal variations in the ratio of lignin to Cr_2O_3 in the faeces of cows. This means that for comparisons between cows by the marker method the cows should be fed at the same times each day, and the faeces likewise sampled at the same time each day. This was the case in the experiments of Chanda, Clapham, McNaught & Owen (50,51). The observation of Kane *et al.* (49) probably explains the apparent alternation of synthesis and destruction of carotene in the gut of the sheep reported by McGillivray (52). It also confirms the findings of Balch (53), Balch & Johnson (54) and Balch & Kelly (55), who worked with roughages to which known amounts of dyed straw of various sizes had been added. It would appear, as might be expected, that the ruminant gut, and no doubt the gut of other species, has an elutriating effect on food particles such that the probable sojourn in the rumen will be longer for a larger particle than for a smaller one and will be longer for a heavier particle than for a lighter one, as Balch & Johnson (54) have found. This property of rumen contents doubtless explains the reduction of milk fat which results when cows are fed insufficient roughage (56,57), and also the fact that such an insufficiency is not overcome by feeding extra roughage which has been ground to too short a length. Such conditions shorten the sojourn of the roughage in the rumen and so shorten the duration of its bacterial digestion. The supply of acetic acid resulting from the breakdown of cellulose by bacteria will be curtailed, so reducing an important precursor of milk fat. Folley and his collaborators (58) have shown that about half the fat in a cow's milk can result from acetic acid reaching the mammary circulation.

Axelsson & Eriksson (59), in a searching investigation of the Cr_2O_3 method, which has been used for years in Sweden, conclude:

(1) That 2-day subperiods are too short for valid comparisons, which presumably implies that the longer the period the greater the accuracy as Chanda *et al.* (60) found.

(2) That with any given dietary constituent accuracy of estimation of digestibility decreases as the amount of it in the diet decreases. A conclusion from this finding is that it should (unless particular attention is paid to mixing) be difficult with the method to determine digestibilities of trace constituents of the food such as carotene. Chanda *et al.* (60) found this also.

(3) That the biggest source of variance is between laboratories. This is probably a consequence of the second conclusion, mixing being more carefully done in one station than another. Crampton & Lloyd (61) used Cr_2O_3 for digestion studies in sheep, and Jarl (62) with cattle. Corbin & Forbes (63) used dye, Forbes protein (64), Forbes & Garrigus (65) lignin, and Homb & Breirem (66) faecal nitrogen in digestion studies in ruminants. Cook & Harris (67) compared the lignin and the natural chromogen techniques.

Sulphur and sulphur-containing amino-acids

Thomas, Loosli, Williams & Maynard (68) have continued their experiments on the feeding of lambs on a synthetic diet devoid of B vitamins and devoid of protein. It contained only cornstarch, corn sugar, cellophane, minerals, lard and urea. Growth on this diet was better than that produced by one in which casein replaced the urea. When sulphate was omitted from the salt mixture of the urea diet for 80 days lambs lost weight but started to gain weight when sulphate was again fed. When the rumen contents of these animals were examined by Gall, Thomas, Loosli & Huhtanen (69) they were found to present microbial facies which varied with the diet in a remarkable way. Cellulolytic organisms were not abundant, most of the organisms being free in the rumen liquid. The predominant organisms in the sulphur-free rumens were facultative anaerobes, while in those of the urea and the casein animals obligate anaerobes predominated. In the microbiological facies of the animals not receiving sulphate, Gram-negative organisms predominated, while in the other two groups Gram-positivity was the rule. Variations of the power of the rumen contents to synthesize the B-complex were also noted in these experiments. The synthesis of the sulphur-containing amino-acids, cystine and methionine from sulphate has been studied by Block & Stekol (70) who fed $Na_2{}^{35}SO_4$ to a cow and obtained, by paper chromatography, radioactive cystine and methionine in the milk proteins. In further experiments (71) Block, Stekol & Loosli fed $Na_2{}^{35}SO_4$ to a milking goat. Precipitation of the milk with trichloroacetic acid showed that 80% of the activity of the milk was in the precipitate and only 20% in

the filtrate. In both the serum albumins and in the milk proteins the activity of the cystine-S was equal to that of the methionine-S. In milk filtrates the radioactivity of the fractions of S showed that SO_4'' > ester SO_4 > neutral S. By contrast, in milk protein hydrolysates 91% of the activity was in the sulphate-free filtrate. The authors interpret these experiments as evidence of the origin of milk protein from the protein of rumen bacteria. Probably this is the correct interpretation, but the possibility of exchange reactions taking place *in vivo* which, in the absence of appropriate enzymes, do not occur *in vitro*, should not be overlooked. The equality of the activities of the cystine and methionine sulphur in these experiments is surprising in view of the demonstration in the rat that cystine can arise from methionine but methionine not from cystine. Possibly the rumen micro-organism build cystine and methionine at equal rates. Even in non-ruminants, according to Layton, Frankel & Scapa(72) inorganic sulphate can be used, for it is incorporated into chondroitin sulphate in the cartilaginous skeleton of the foetus of the rat. The same authors assert that sulphate is incorporated into the connective tissues of the chicken. Since chickens need chondroitin sulphate as a growth factor this is very interesting. Klosterman, Bolin, Lasley & Dinusson(73) have shown increased retention of absorbed N from 13 to 18% by sheep when a ration containing 30% of field peas as the chief source of protein is supplemented by methionine, thus again demonstrating the relative poverty of pulses in this amino-acid. Yakovlev, Odinets, Ozerova & Kanygina(74) found keratin, in spite of its richness in S, to be a worse wool producer than cotton seed when added to the diet.

VOLATILE FATTY ACIDS

At the time the previous review(75) was written there was still some doubt as to the relative importance of volatile fatty acids (VFA) and carbohydrate in ruminant nutrition. Recent work would seem, however, to confirm the prognostications of earlier workers in this field. Such early prognostications were made by workers in Germany whose work was reported by Mangold(76). Marston and his colleagues had long suspected their importance, and the workers at Cambridge with the late Sir Joseph Barcroft produced quantitative proof of their importance. It was, however, the development of new chromatographic techniques under the stimulus of Martin and his colleagues(77) that led Elsden(78) and later Moyle, Baldwin & Scarisbrick(79) to design reliable methods for characterizing the VFA which arise from fermentation in the rumen and which form an important constituent of the blood of ruminants. In the previous review by the present author it was stated that the tissues of the ruminant seemed to have evolved in adaptive relation to the fermentation occurring in the rumen. This hypothesis is still the most convenient for correlating the rapidly increasing body of facts about ruminant nutrition and physiology. Thus the adult ruminant has a peculiarly low blood sugar and a blood VFA titre which is much larger than those of man or dog(80). In spite of the extremely low fat content of its habitual food the ruminant is prone to ketosis (see later for references to this). Ruminant mammary tissue is much superior to mammary tissue of non-ruminants in using acetic acid as a metabolite for the synthesis of fatty acids particularly the lower ones(81,82,83,84). As a result of this latter peculiarity the ruminant's milk has an outstandingly large Reichert-Meissl value(85) which diminishes during starvation(81), as arterial titres of VFA diminish(81). Bound up also with this peculiarity is the ruminant's exhibition of a respiratory quotient in actively lactating mammary tissue, greater than unity(75,83,86). The synthesis of fat in the ruminant's mammary gland is, unlike that of other species tested(82), insensitive to increase by the action of insulin *in vitro*. Peculiar to the ruminant also is the power of the older or adult animal but not of the suckling to dispense with the so-called indispensable amino-acids and the vitamins of the B-complex. Finally, in this probably incomplete catalogue, there is the ruminant's large requirement of cobalt which other animals seem to need only as the cobalamin, vitamin B_{12}.

All these peculiarities would seem best considered as by-products or consequences of its peculiar microbiological method of utilizing nature's most abundant living source of energy, cellulose. This method is a perfect example of obligate symbiosis for the micro-fauna and micro-flora of the rumen cannot persist long without the rumen host. The host, likewise, soon becomes

very sick if its rumen microbiology is extensively disturbed. Such disturbances as ketosis (see section on this subject) and bloat are part of the price which the ruminant pays for being able to manufacture its own amino-acids, water-soluble vitamins and fatty acids, and for being able to digest the polysaccharides such as cellulose, hemicellulose and certain other constituents of the cell-walls of plant materials. McClymont(87) and Reid(88) have shown that in the peripheral circulation of the sheep and the cow the volatile fatty acids resemble those produced by the bacteria in the rumen in not being esterified, but differ markedly from the latter in the ratio of one to the other. Thus in the rumen acetic and propionic preponderate, butyric comes next, followed by much smaller amounts of formic acid and of acids of carbon chain lengths longer than butyric(89,90). Thus the partition of the total acidity between the various acids changes as a result of absorption. Masson & Phillipson(91) analysed rumen portal blood of sheep with the rumen isolated by ligatures from both anterior and posterior portions of the gut and made accessible by a rumen fistula. Mixtures of acetic, propionic and butyric acids were placed in the previously emptied rumen of the animal (which was still anaesthetized with intravenous Nembutal). Acetate, propionate and butyrate were all absorbed in substantial amounts through the rumen wall. The order of absorption rates was: acetate > propionate > butyrate, but the concentration of the acids in the blood from the rumen veins was not proportional to the rate at which the acids disappeared from the rumen. Moreover, although the acetic acid in arterial blood reflected that in the rumen veins, this was not true of the other two acids. It was obvious that not only the liver but also the rumen wall participated in some kind of active metabolism of butyric and propionic acids, and in partial support of this hypothesis it was found that as volatile fatty acid was absorbed through the rumen wall it was in part replaced by incoming chloride and bicarbonate. Phillipson's colleagues followed up these clues. Parthasarathy(92) and Garton(93) further investigated the mineral metabolism of the rumen wall. By Warburg manometry Pennington(94) showed that butyrate, and to a lesser extent propionate and acetate, are metabolized by the rumen epithelium. Butyric acid (but not propionic) was converted to ketone bodies (mainly aceto-acetic acid). Acetic acid formed some ketone bodies but not to the same extent as did butyric. Thus Masson & Phillipson's results were explained in so far as butyric is concerned. The utilization of propionic was improved when 5% CO_2 was present in the flasks. Butyric produced ketone bodies in the presence of reticular and omasal tissues. Abomasal and caecal epithelium did not show a preference for butyric and produced relatively less ketone bodies. Later Annison & Pennington(95) showed ketone body production by sheep's liver but not by sheep's kidney from *n*- and isovaleric acid. Schambye & Phillipson(96) had earlier stressed the importance of VFA in sheep when they showed that differences between rumen venous and carotid blood were much bigger for VFA than for glucose. Schambye(97,98,99), continuing these studies, has found that introduction of sugar into the rumen produced only transient rises in portal and carotid blood glucose titres in normally fed sheep. The rise of blood glucose was greater on diet of hay alone than on one of hay plus oats(99). Glucose had an enhanced effect on blood glucose after a 3-day fast when presumably the rumen bacteria would have declined in numbers and perhaps in fermentative activity. On hay alone(98) the arterial portal differences of glucose did not change, though the VFA increased by a third 1 hr. after eating. Blood sugar was not affected by adding 100 g. crushed oats to the diet. Two days' starvation had little effect on blood sugar, but the arterial and portal VFA fell until by the fifth day they were negligible. Hepatic venous blood(97) showed no change when acetic acid was placed in the rumen, thus demonstrating the power of the liver to remove it from circulation. During digestion of a diet of chopped hay, linseed and oats(97) the ratio of acetic to propionic to butyric in the rumen was 75:14:11 and arterial and portal blood sugar were unchanged. Kiddle, Marshall & Phillipson(100) showed that blood draining the rumen was deficient in butyric acid by comparison with the mixture of acetic, propionic and butyric placed in the rumen. In the same experiment they found lymph to be unimportant as a vehicle for VFA. Johnson(101) found the order of absorption of fatty acids from the rumen to be butyric > propionic > acetic in agreement with Phillipson's school. He further suggested that an excessive production of butyric acid from the rumen and

a shortage of glucogenic substances might be a cause of that form of ketosis in ruminants which is curable either by injection of glucose or by injection of adrenal cortical extracts. Such extracts produce protein katabolism and thus release amino-acids into the system, some of which are glucogenic. Jarrett & Potter(102) showed that in a phlorhizinized sheep injection of acetic acid was ketogenic, but that the excitement caused by intraperitoneal injection of acetate, normal saline, or glucose itself caused a delayed glycosuria so that no conclusion could be drawn from the experiments. Reid(88, 103), like Schambye, found a fall of blood VFA during fasting. VFA reduction of jugular as compared with carotid blood was all accountable to acetic acid which formed 90% of the circulatory VFA. Arterio-venous differences of VFA persisted on a variety of diets and were always greater than the differences of glucose, which were, nevertheless, not themselves negligible. Glucose presumably results from digestion in the small intestine by pancreative juice and succus entericus, of carbohydrates which have escaped change in the rumen. Polysaccharides of iodophilic microflora and oligotrich protozoa(104), and the glucosan demonstrated in the holotrich protozoa by Oxford(105) and by Masson & Oxford(106) will presumably also contribute to glucose arising from what might be called 'succal' digestion to distinguish it from digestion resulting from symbionts in the rumen or other parts of the alimentary canal. It is convenient at this point to stress a distinction, drawn by the reviewer in earlier reviews(75,107) in this series, between animals like the average sheep which spends a considerable portion of its life on a plane of nutrition not far above maintenance and such an animal as a cow yielding over 4 gal. of milk per day. Much (and probably most) of the starch and protein in the grain fed to the latter animal will pass on so rapidly to the abomasum that most of the digestion will be succal in type. It is probably for this reason that urea is still extensively used to supplement corncobs and other maize products in producing beef in the corn belt in U.S.A., while New England dairy farmers in the U.S.A. do not use urea. Urea is of no use as a major source of protein to an animal with a succal digestion. It was no accident that the Germans demonstrated the utilization of urea in lambs long before they could obtain any unequivocal effects with lactating cattle(108). Gray, Pilgrim & Weller(109), comparing the production of acetic, propionic and butyric acid in the rumen of sheep eating either wheaten hay or lucerne hay with the production of the acids when rumen contents of the same animals were incubated *in vitro*, found that the ratio of propionic to acetic was much greater *in vitro* than *in vivo*. They, therefore, concluded contrary to Masson & Phillipson(91) that propionic is absorbed faster than acetic at the acid pH which normally obtains in the rumen. An interesting feature of the studies of Gray *et al.*(109) was that, using ammonium carbonate instead of calcium carbonate as a buffer, 40% of the cellulose of wheat or lucerne hay was digested *in vitro* in the short space of 2 days, cellulose being determined chemically by the method of Norman & Jenkins. Even greater percentages of the free pentosans were digested at the same time. Methane was evolved in natural amounts, and at the end of the 2 days the protozoa were still alive. In a later paper, Gray & Pilgrim(110) found with wheaten hay that during the first $4\frac{1}{2}$ hr. of incubation *in vitro*, propionic acid was produced fastest, thereafter acetic was fastest after which the rises of acetic, propionic and butyric were each linear with acetic acid still the fastest. Later still, Gray, Pilgrim, Rodda & Weller(111), working with carboxyl-tagged acetic acid, found that radio-acetic gave rise, when incubated *in vitro* with rumen contents, to radio-butyric, radio-valeric and radio-propionic. Radio-propionic produced radio-valeric and a trace of radio-acetic but no butyric. In these experiments, by using the method of Moyle *et al.*(79) on columns of buffered celite (diatomaceous earth), Gray *et al.*(89) demonstrated the presence of formic, acetic, propionic, butyric, isobutyric, valeric, caproic and a heptoic acid. Formic acid has been repeatedly demonstrated in the rumen. Methane of the rumen seems to arise from microbial reduction of CO_2(112,113). The intravenous injection of VFA has been studied(103,114,115). Reid(103) has found that propionic acid relieves the hypoglycaemia induced by insulin in adrenalectomized sheep, almost as rapidly as does glucose itself. Reid, in the same paper, points out that VFA in the blood is much higher in ruminants than in non-ruminants, and that in the sheep at the height of post-prandial rumination acetic forms 86–95% of both the carotid and jugular VFA. The

possibility that there is any mechanism other than fermentation which maintains the level of circulating acetic acid in ruminants would be worth investigation, even although the low levels engendered by starvation (98) argue against such a possibility. Injection of propionate but not of acetate (114) into sheep increases blood glucose, pyruvate and lactate. Propionate injections depress but acetate injections increase blood ketones. A mixture of propionate and acetate does not increase blood ketones. The authors suggest that the ratio of acetate to propionate in the VFA arising in the rumen may be related to the onset of ketosis. Propionate (115) disappears from the blood after intravenous injection faster than does either acetate or butyrate. Jarret & Potter (116) have studied the neonatal VFA and sugars in the blood of lambs and have compared them with those of sucklings and weanlings. Neonatally, blood sugar was 110 mg./100 ml. of which 60 mg. was fructose. Only 5 hr. post-partum fructose had dropped to 4–8 mg./100 ml. When the lambs suckled there was a rise in blood glucose and a transient rise in blood pyruvate. For 2 weeks glucose was 120 mg./100 ml., but thereafter it gradually fell to the adult level. Hyperglycaemia from intravenous glucose persisted longer in adult sheep than in lambs. The total VFA in the lamb's systemic blood was of the same order as in adult sheep 2–15 ml. N/100 acid/100 ml. Phillipson (117) found that lambs maintained on Stewart's (118) cobalt-deficient diet developed unusually acid rumens and had poor appetites. Lactic acid appeared in more than usual amounts in the rumen (7 m.equiv./100 ml.) and more propionic and less acetic than usual were found. Adding glucose to the rumen favoured greater production of lactic acid. Free and esterified VFA in the hair-grease of man, horse, rabbit, cat, guinea-pig, rat, goat, cattlebeast and sheep have been measured by Brouwer & Nijkamp (119). In the three ruminants seven VFA were present (C_2, C_3, C_4, C_5, C_6, C_8, C_{10}). In cattle and male goats C_8 to C_{10} acids preponderated. In dogs valeric was present by itself. The authors question whether the smell of male goats is due to C_6–C_8 acids as is sometimes asserted. The reviewer once thought that the characteristic odour of the male goat was in its urine, but uncontaminated urine from male goats has no smell. In the reviewer's opinion the odour may be connected in some way with the male sex hormone, for adult male castrates have not the odour (120).

Masson & Phillipson (121) found the digesta leaving the abomasum of the sheep contained up to 20 times less VFA than did the contents of the rumen. At the same time concentration of chloride increased from rumen to omasum and omasum to abomasum. Gastric juice in surgical pouches had 157 m.equiv. chloride/l. during feeding or 153 during fasting. The greatest acidity was 170 m.equiv./l. Gastric acidity was reduced during fasting. From considerations of chloride content Masson & Phillipson deduced that omasal material is diluted an equal volume in the abomasum.

FAT IN THE DIET AND MILK FAT

Dijkstra (122) compared the milks of cows receiving 5 kg. concentrates plus 69 g. fat with milk of cows receiving the same concentrates plus 194 g. fat. The group with the higher fat intake produced slightly less milk somewhat poorer in non-fatty solids, somewhat richer in fat. The same author (123) compared the effects on milk of pressed and solvent-extracted cattle cakes. The pressed cake produced more milk without increasing the fat percentage and without affecting the weights of the cows. Volcani (124) states that the alleged effect of citrus fruit on milk fat is really probably a climatic effect. Jimenez-Diaz, Marina & Romeo (125) report, in experiments on six dogs of which the bile ducts were ligatured, that although such ligaturing produced steatorrhoea while the animals were on a basal diet, there was no evidence that their digestion of added fat (50 ml. olive oil) was impaired. The calorific value of the faeces was nevertheless increased.

KETOSIS

Blood sugar of ruminants

Reid (126, 88) has made a study of the blood sugar of sheep in relation to diet in both pregnant and non-pregnant animals. He has reviewed (126) the work of various authors who measured blood sugars, and states that when methods which fail to distinguish sugars from other reducing sub-

stances in the blood are excluded, then it is true to say that ruminants have a characteristically low blood sugar. In pregnant ewes, poorly fed, the blood sugar is even lower and drops to values as low as 9–32 mg. % when pregnant ewes are starved for 24 hr. Low blood sugars are accompanied by large increases of ketones in the blood. Indeed Snook(128) found that pregnancy toxaemia in ewes is associated with a marked ketosis cured by feeding starch. Reid(88) asserts, from his experiments, that the low level of blood glucose of the sheep is accompanied by a smaller uptake of glucose by the tissues, and that this low uptake is a reflex of the greater propensity to metabolize acetic acid. Reid thus agrees with Dye & McCandless(129) and disagrees with Mann & Zarrow(130) about peculiarity of ruminants in respect of the concentration of sugar in the blood. Reid(131), in further studies of arterio-venous differences, found sheep to be slow, by comparison with other species studied, in removing circulating glucose either intravenously injected or introduced into the abomasum. Holmes(132) produced hyperglycaemia in cows by intravenous glucose 0·27 g./kg. body weight in 18% aqueous solution. Blood values returned to normal after 2½ hr. in normal cows but took longer in cows from which food had been withheld for 2 days. Lesbouyries & Charton(133) point out that ketosis is a more serious condition in sheep than in cows. This is probably due to lesser incidence of periods of undernutrition and of multiple pregnancies in cattle (Snook(128)). Ketosis in 200 of 600 cows was found by Holmes(134) to be associated with undernutrition in late winter and to disappear when the animals went to grass again. McEwen & Foggie(135) found that wintering in byres was more conducive to ketosis in cattle than wintering in courts. Shaw(136), in comparing cows on a carotene-deficient diet and cows on the same diet supplemented with vitamin A, could find no evidence of any connexion between vitamin A deficiency and ketosis, in spite of the development of obvious signs of vitamin A deficiency in the calves of the deprived cows. Ketones and sugar in the blood of the A-deficient dams were normal. Seekles(137) reports successful treatment of ketosis in cattle with 120 g. commercial ammonium lactate (80–85%) dissolved in a pint of water or mixed with the feed. He found it useful in curing ketosis resulting from the ingestion of too much butyric-rich silage. The rationale of this treatment was that post-mortem examinations of livers of ketotic cattle showed the liver to be both fatty and deficient in glycogen which the liver can form from lactate. Carlström & Carlström(138) and Carlström(139) state that in ketosis the adrenal metabolism is faulty and that consequently K in the blood is subnormal. They found that injection of extracts of adrenal cortex, of deoxycorticosterone, of creatine plus adenosine triphosphate or of KCl afforded relief. Carlström(139) again calls attention to the distinction between 'small farm' and 'estate ketosis', the former due to underfeeding, the latter to imbalance of nutrients. Talsma(140) claims cures of post-partum ketosis in milch cows with $KClO_3$ (70 g./day for 4 days) but not with KCl. Saarinen & Shaw(141) have made a study of the biochemistry of ketosis in cows. A post-mortem finding was flabbiness of the adrenals. Distribution of Na and K in the blood was normal but that of lipids was disturbed. Blood ascorbic acid was very variable. Shaw, Hatziolos & Chung(142) claimed improvement of ketosis by injection of either cortisone or adrenocorticotrophic hormone, but the improvement could not be directly related to either blood glucose or blood ketones. Sandstedt, Dyrendahl & Hjalmars(143) incrimated Mn in ketosis when they found it in cattle grazing an area in which oat crops showed the leaf markings of Mn deficiency. If this is indeed the case, then overliming of pasture might perhaps be involved(144). Schultz & Smith(145), in confirmation of earlier workers, found that in ruminants (female goats) the diabetogen alloxan increases both blood glucose and blood ketones, whereas insulin depresses glucose in the blood. It is convenient to note here that in the foetal and neonatal lamb that part of the blood sugar which is contributed by fructose originates(146) from the power of the placenta to convert glucose to fructose.

In cows suffering from ketosis, Robertson, Thin & Stirling(147) have demonstrated appreciable amounts of isopropyl alcohol in blood, milk and rumen contents. It may be an important precursor of ketone bodies.

In later papers Holmes(148,149) has studied the physiology of ketosis in cows in more detail. In cows suffering from ketosis(148) the tolerance of intravenous glucose is unaffected in spite of

the increased amount of ketones in the blood. By analogy with dogs, cats and man it might be expected that oral glucose would decrease the titre of ketones in the blood of cattle. That the reverse is the case in normal cattle is shown by Holmes(149) for oral glucose in doses of 2·24–8·94 g./kg. produced acetonuria after 1–5 hr. In the young ruminant (less than 1 year), however, the expected response was found, i.e. no acetonuria followed and the blood sugar was doubled. In three of the five cows in which acetonuria was observed after oral glucose, the rises of blood glucose were much smaller than the rises observed in the younger animals. Since there was no acetone detectable in rumen contents, it is fair to agree with Holmes that the ketone bodies originated in the liver from VFA produced in the rumen from the glucose. Coop(150) found that the ability of sheep to ferment glucose in the rumen diminished in starvation but returned within 12–24 hr. of re-alimentation. Quin, Oyaert & Clark(151) found that the cellulolytic power of the rumen of sheep was likewise diminished considerably by a fast, while at the same time its contents became very watery. The fluid percentage increased from 43 to 60% in spite of the fact that the sheep drank less water when fasting. In these experiments the recovery of both the appetite and the power of the rumen to digest cellulose were more rapid in sheep which were eating hay than in those which were eating lucerne before and after the fast. The authors attributed these differences to contamination of the hay with organisms which normally thrive in the rumen but did not investigate the possible importance of the relative recuperative power of already established flora of the rumen. These re-alimentation effects were not so marked in cattle.

McCandless & Dye(152) have made a study of the blood sugar of domestic and wild ruminants both young and adult, and have also studied the sugar metabolism of camels and llamas. They confirm that in ruminants generally (including the cow, sheep, goat and several wild forms) the blood sugar of the young is of the same concentration as is found in carnivores, but that with the onset of active rumination the blood sugar falls and remains at the lower adult level. At the same time the tolerance of intravenously injected glucose also falls, so that the young ruminant has better tolerance than the adult. No convincing theory as to the cause of this change in ruminant metabolism with age has yet been advanced.

MOVEMENTS OF FOOD IN THE ALIMENTARY TRACT

The search for specific functions for different parts of the alimentary tract has prompted renewed investigation of the rate of passage of food through various parts of the gut, though no one seems to have X-rayed the intact sheep, calf or adult cow after marking the food with $BaSO_4$ or iodine, which would ensure that the animal's condition was very near normal. Such methods would not, of course, permit the chemical analyses of digesta passing various points of the gut such as were made by Masson & Phillipson(121). Comline & Titchen(153) have studied the action of the oesophageal groove in calves up to 14 days old. They used 'decerebrate' preparations. The superior laryngeal was the only nerve which consistently caused the groove to close, but the entry of water into the posterior part of the mouth was an even more effective stimulus. The efferent fibres were largely confined to the dorsal abdominal vagus nerve. Reflex contraction of the groove was associated with swallowing but still occurred after buccopharyngeal movements had been abolished by section of the appropriate nerves. Stimulation of the central end of the glossal branch of the glossopharyngeal nerve inhibited swallowing, respiration and reflex contraction of the groove. Recovery in all three was rapid on cessation of the glossal stimulus. Stimulation of the central end of the abomasal nerve inhibited reflex groove contraction but not swallowing or respiration. No evidence was found of inhibition of groove musculature by sympathetic innervation. In these decerebrate animals solutions of copper salts were no more effective than water in causing reflex closure of the groove. Phillipson(154), by operative techniques, measured the rates of efflux of digesta from the sheep's abomasum throughout the day. Hay passed more slowly from the rumen than did meals, and during rumination the flow from the abomasum was more regular than at other times. Digesta passed to the duodenum in gushes at irregular intervals for 17 out of the 24 hr. Such gushes may cease for intervals of 15–30 min.

especially after hay has been eaten. Duncan & Phillipson (155) have studied the development of the suckling reflex response in foetal sheep. As early as 80–100 days the reflex pattern may be present, and at 140 days the suckling is vigorous. Full development of the stomach with innervation took place between the 26th and 55th days. Duncan (156) finds that the movements of the reticulum in normal sheep are diphasic, rapid and systolic, and thinks their extreme regularity indicates that they originate in the brain. Iggo (157) finds that the double contraction of the reticulum precedes a contraction of the rumen. He also postulates a central origin for these contractions. Singleton (158) has adduced evidence that abomasal contractions are inhibited by an acid pH in the duodenum or by the presence in the duodenum of the products of digestion of fat, protein or carbohydrate. Clark & Weiss (159) have demonstrated reflex salivation in the sheep and the goat by stimulation of the cardiac area of the rumen. When the vagi were sectioned stimulation of the central end of either the right or left vagus caused salivation. Either urea (160) or hydrocyanic acid (161) causes ruminal paralysis. Possibly the former acts through causing a sudden increase of rumen pH. Urea toxicity was increased by alkali and decreased by acetic acid or by a bigger protein intake by the animal.

Balch, Kelly & Hein (162), by means of balloons inserted through a rumen fistula and attached to a kymograph, showed that during resting, eating, ruminating, lying or drinking or while the cow was being milked the reticulo-omasal orifice functioned in a constant pattern. For the greater part of the reticulo-rumen cycle of contraction the reticulo-omasal orifice was open, but following the last reticular contraction it closed strongly. The last reticular contraction accompanied a fall of pressure within the omasum. This fall of pressure was followed by a closure of the reticulo-omasal orifice and then by a rise of the pressure within the omasum. Balch *et al.* considered that passage of digesta from the reticulo rumen is relatively continuous throughout each 24 hr. and that its rate is governed by the valve-like action of the omasum. Kesler, Ronning & Knodt (163) weighed the digesta and tissues of fifty-nine calves, aged 8 days to 16 weeks. Rumen contents increased from 0·01 lb./lb. body weight at 8 days to 0·1 lb./lb. at 16 weeks. Rumen tissues increased from 0·005 to 0·02 lb./lb. body weight at 8 days and 16 weeks respectively. Trautmann & Hill (164) found the difference of temperature between the abomasum and the rectum to be $\pm 0{\cdot}1°$ C., which in turn was 2–4° C. higher than the temperature in the rumen. Consumption of hay increased the rumen temperature by 2° C. Drinking of water caused it to fall. Blamire (165) has reported new data on the capacities of various bovine 'stomachs'. Stoddard, Allen, Hale, Pope, Sorensen & Winchester (165) have described a rumen fistula fitting made of Perspex (lucite). New apparatus for collection of excreta from ruminants is described by Bratzler (167) for sheep and by Balch, Bartlett & Johnson (168), by Richter & Becker (169) and by Hobbs, Hansard & Barrick (170) for cows and steers.

Garton (93) has studied the distribution of dry matter Ca, P and Mg throughout the gut of the sheep. In the rumen P is enriched by P from saliva while Ca and Mg arise from rumen digestion. Dry matter, Ca, P and Mg concentrations in the omasum lend support to the hypothesis that it is a water-absorbing organ. In the abomasum Ca seems to arise from some, as yet unidentified, compound.

Using radio-P, Scarisbrick & Ewer (171) found that the rumen wall was permeable in either direction to phosphate but that over a long period there was no evidence of net absorption. Presumably phosphate will be rapidly incorporated into the rumen bacteria and protozoa. Parthasarathy (92) also found no net absorption of phosphate. He found Na^+ to be absorbed against a greater concentration in the blood and K^+ to be absorbed because of its greater concentration in the rumen. Chloride and CO_2 passed into the rumen through the wall, but in the opposite direction to the VFA. Parthasarathy's results and those of Sperber & Hyden (172) are at variance, possibly because of the difference in physiological states of the animals studied. Thus the latter found the concentration of both phosphate and K in the blood to exceed the concentration in the rumen liquor, and found that chloride in blood exceeded chloride in rumen liquor. In a rumen pouch chloride was absorbed against the gradient while the K in the pouch increased. Evidently osmotic conditions in the rumen wall are too complex to be understood by

a simple inorganic analogy. Parthasarathy(92) found absorption of Na and K in the small intestine and of chloride in the small intestine and caecum. Johns(173,174,175) has isolated from the rumen of sheep an organism which produces propionic acid from lactate or succinate. When it produces propionate from lactate CO_2 fixation occurs so that the yield of propionate depends upon the partial pressure of CO_2. The organism was a strict anaerobe and this property and its biochemical behaviour enabled Johns to identify it with *Veillonella gazogenes*. Hungate(176) has isolated an obligate anaerobe (*Bacteroides succinogenes*) from the rumen. It is a cellulolytic rod. It produces acetic, succinic, propionic and butyric acids from cellulose or starch. It is Gram-negative and non-motile. In the course of these experiments another cellulose-digesting organism was also isolated. Sijpesteijn(177) has isolated two strains of a cellulolytic bacterium (*Ruminococcus flavifaciens*), one from sheep, the other from cattle. They are anaerobic Gram-positive streptococci which attack cellulose and cellobiose but not starch, maltose, lactose or xylose. One of the strains could use glucose, the other not. On media containing cellulose the organisms produced a yellow pigment. With cellobiose this pigment was absent. Growth on cellulose was favoured by the presence of *Clostridium sporogenes* or of sterilized medium in which the Clostridium had grown. Its fermentation products were succinic, acetic and formic acids. It would appear from studies on the rumen by Sijpesteijn & Elsden(113) that the succinate in the rumen is readily converted by the bacteria into propionate. Pursuing further studies, which have already been mentioned(105,106) Sugden & Oxford(178) have described the culture *in vitro* for periods of a month of the holotrich ciliate protozoa of the sheep's rumen and have extensively studied their fermentative abilities. Grass juice was essential for their long survival but boiled grass juice was also partially effective. Hobson & MacPherson(179) have reported the occurrence of capsulation in rumen bacterial centrifugates, but it is not clear what relation this phenomenon bears to the zooglaea formation reported by Smith & Baker(180). Work on the organisms associated with the peculiar biochemical properties of the rumen still continues. Thus Karunairatnam & Levvy(181) have found glucuronidase activity in the rumen and have associated it with the medium-sized micro-organisms in the rumen but not yet with any well-defined species. They suggest that the real function of this enzyme may be to potentiate the bacterial breakdown of hemicelluloses in the rumen. Baker, Nasr, Morrice & Bruce(182) have compared the digestion of starches and starch products by ruminants and non-ruminants. In animals with diverticulated alimentary tracts starch grains are digested in these diverticula, whereas in other animals such starches are digested by the animals' own digestive secretions. This paper should be consulted for photomicrographs of starch being attacked inside the host by symbiotic bacteria. Other illustrations of similar phenomena are given by Masson(183) in a review. Van Der Westhuizen, Oxford & Quin(184) have, by implication, agreed with Ingram & McGaughey(185) that *Schizosaccharomyces ovis* Quin was misnamed for they found it irresponsive to culture methods suitable for a yeast, though Van Der Westhuizen *et al.*(184) do not go so far as to assert the organism is a protozoan (see previous review, Owen(107)). Nasr(186) could not find free amylase in the rumen of sheep, nor did its saliva contain any. Nasr noted the presence in the rumen of *Clostridium butyricum* which produces an amylase in pig's caecum(182). Conrad, Hibbs, Pounden & Sutton(187) found that, as long as they were receiving milk plus alfalfa hay to appetite, calves which had been given access to cud from older animals were better digesters of cellulose than calves not allowed such access. The difference disappeared when the milk was discontinued and the animals received only alfalfa or lawn clippings. The difference was attributed to the presence of protozoa. Two calves were given a diet of pure grain till the microbial facies had changed. On switching them back to the alfalfa regime one of them digested cellulose better than the other. This better digester was found to have acquired a characteristic microbiological facies. Pounden & Hibbs(188) reared calves free from protozoa and with a different microflora from the usual one. Contrary to earlier reports by other workers, Pounden & Hibbs found that calves with the protozoa in the rumen were more thrifty than those without. Heald(189) has studied the fermentation of pentoses and uronic acids by five strains of Gram-negative coliform rods from the sheep's rumen. Using xylose, glucuronic acid, glucose and cellobiose as substrates, he found

formic, acetic and lactic acids, ethanol, butylene glycol and acetoin among the end-products with formic and acetic acids predominating. No lactic acid, butylene glycol or acetoin came from xylose and neither glucuronic acid nor cellulose gave rise to ethanol. Heald(190), in an earlier paper, has studied the glucose-containing substances of the rumen of sheep and finds glucose to form some 4 or 5% of the dry matter of the micro-organisms. Reducing substances in the rumen are, he shows, only in part attributable to glucose. Glucose-containing carbohydrates in the rumen micro-organisms increase markedly after feeding and then rapidly decline. Burroughs, Gall, Gerlaugh & Bethke(191) found that in a ration of corncobs, alfalfa hay, starch, minerals, and vitamins A and D, the digestion of roughage was facilitated by casein. Omission of the casein reduced the digestibility from 53 to 13%, and the bacterial count from 48 to 25 thousand millions per gram. Digestion of roughage was unimpaired by omission of both the starch and the casein and in these circumstances the bacterial count was hardly changed (50 thousand million per gram). The same workers(192,193) have studied the digestion of cellulose by the rumen microflora *in vitro* by their continuous transfer technique in which after every 36 hrs. the culture is diluted with fresh nutrient medium. By this technique up to 90% of added cellulose is digested by rumen bacteria. They found that a complex salt solution or a solution of alfalfa ash, or autoclaved rumen liquid or autoclaved extract of manure of the same animal as that from which the rumen material came, would potentiate cellulose digestion *in vitro*(192). Various foods(193) were compared with the most stimulating addition, manure extract, as potentiators of cellulose breakdown *in vitro*. Of eleven foods tried skimmed milk was best and liver was the worst with various concentrates of plant origin in between. Meites, Burrel & Sutton(194) have investigated the activity *in vitro* of rumen cellulase. Like Pochon(195) they found cellulose could be digested *in vitro* in the presence of toluene, with the simultaneous production of reducing substances. Of these they separated three chromatographically and showed that one of them was glucose. Berkefeld filtration of rumen contents failed to reveal any soluble cellulase. Michaux(196) found a large digestibility of pectic substances by sheep and lambs from hay, apple pulp, beet pulp and straw. He also made simultaneous measures(197) of the digestibility of cellulose in these same materials.

The great variety of organisms present in a healthy rumen continues to present a formidable challenge to microscopists, bacteriologists and biochemists. Like those of the soil, its flora and fauna present a complexly interacting community. The vast variety of types of organisms still to be characterized is suggested rather than proved by many authors(198,199,200,201). Moir & Williams(198) experimented in Western Australia with five sheep, each of which received five different diets in random succession. The diets contained five different levels of protein ranging from 3 to 12%. Rumen bacteria were sampled by stomach tube, a previous experiment on animals which were subsequently killed having shown that a stomach tube gave a representative sample of rumen microbial contents. Moir & Williams found a correlation of $r = +0{\cdot}98$ between the protein intake of the sheep and their bacterial population which varied from 25 to 64 millions per cu.mm. of rumen contents. Moir(199) found a large seasonal variation in the rumen population of sheep on natural grazing in Western Australia, where, on account of the Mediterranean type of climate, the grazings become very desiccated in midsummer. Rumen counts began to fall precipitately in mid-November when the sun begins to scorch the grass. A rapid recovery of numbers of bacteria in the rumen occurred from May to November. Such a study would be even more interesting had a sheep or two on irrigated land been included. In this study the number of protozoa and of *Oscillospira guilliermondii*, the large obvious rumen bacterium were also counted. Their trends were very close to those for total bacteria. Moir & Masson(200), working in Scotland, published an illustrated scheme of classification of rumen micro-organisms (excluding the commoner protozoa) and showed twenty-five photo-micrographs. Thirty organisms are described including *Oscillospira guilliermondii*, *Selenomonas ruminantium*, vibrios, rods, spirals, diplococci, sarcinae and coccoids, some free, some adherent to food particles. Gall & Huhtanen(201) have set up criteria for judging whether an organism is a genuine rumen organism. These criteria are:

(1) The organism must be able to live anaerobically.
(2) Rumen contents must contain more than one million of it per gram.
(3) It must be isolated at least 10 times from at least two animals.
(4) It should be found in animals in at least two different geographical locations.
(5) It should be able to produce the sort of end products found in the rumen.

One may readily object to each of these in turn on a variety of grounds, but with their technique of isolation Gall & Huhtanen needed some criteria for rejecting suspected contaminants and these were the criteria they adopted. Five Gram-positive organisms (1 coccus and 4 rods) fulfilling these criteria are described, and the technique of isolation is fully described in a later paper by Huhtanen, Rogers & Gall(202). With their precautions for obtaining anaerobiosis rumen bacteria may be grown from rumen contents which has reached its last serial dilution at 10^{-11} of the original rumen liquid taken from a fistula or stomach tube. Pounden, Ferguson & Hibbs(203) have extended Baker's earlier studies and tried to trace morphologically those rumen micro-organisms which pass on to the more remote parts of the gut. Protozoa were disintegrated in the abomasum, as also were many rumen bacteria. Among the bacteria all grades of digestibility were found, some being very readily digested, others being morphologically still recognizable in the faeces. A similar study was made by Uzzell, Becker & Jones(204) in respect of the protozoa. At birth the stomach and intestines of the calf were devoid of protozoa as would be expected. Protozoa teemed in the rumen of older animals, but in the abomasum they were non-motile and in the ileum they were all dead and disintegrated. Eighteen species of eight genera of rumen ciliates were identified in twelve Jersey calves and a cow.

Oyaert, Quin & Clark(205) found that sulphanilamide depresses cellulose digestion in sheep and cattle. They also noted a loss of appetite which the present author can confirm for milking cows. They found that rumen fermentation of sugar and gas production were also depressed. These last effects may have been secondary to the loss of appetite, as probably is the temporary depression of milk yield of cows by orally administered sulphonamide.

BLOAT

The etiology of bloat continues to be the subject of new theories or of new revivals of older theories. The theory that an intractable emulsion formed in the rumen is the cause is favoured by Nikitin, Tverdun, Lebedinskii, Locko & Mamina(206), who state that in bloat there is defective mixing of the gut contents, and by Quin, Austin & Ratcliff(207). The latter authors state that of 155 cases, they treated 115 successfully with methyl silicone polymer which, presumably by dissolving natural emulsifiers, raises the surface tension of rumen liquid and so facilitates the normal release of carbon dioxide and methane. Ferguson, Ashworth & Terry(208) isolated from lucerne a crystalline flavone which could inhibit the contractions of smooth muscle. They suggested that this flavone was present in greatest quantity in the lucerne at the stage of growth when the plant was most capable of causing bloat. Hungate, Dougherty, Bryant & Cello(209) carried out experiments on sheep to help to elucidate the problem of bloat. They found that the sudden introduction into the rumen through a fistula of 6 lb. of rye plus 4 l. water caused death. Untoward symptoms were caused by similar treatment with 4 lb. rye or 4 lb. cracked corn or with large quantities of glucose solution. Relief was afforded to a corn-fed animal when the contents of its rumen were replaced by those of a hay-fed sheep. Examination of rumen contents by phase-contrast microscopy and culture methods showed a complete change of the rumen microbial facies in the experimental animals. The protozoa were dead, there was a great diminution of cellulolytic organisms and a very marked increase of *Streptococcus bovis*. The rumen pH was very acid (even as low as 4) and lactic acid was superabundant. Experiments on further sheep in the endeavour to incriminate *Str. bovis* as the cause of the bloating were inconclusive, though counts of *Str. bovis* in affected animals were above normal. In the same paper two cattle dead from gorging apples(209), were examined. Rumen pH was 5, but *Str. bovis* counts were normal. In one sheep, drenched with glucose via the fistula, the glucose in the portal blood rose to

525 mg. % which was not regarded as injurious by the authors. No evidence was obtained in any of these experiments of any specific toxin in the rumen. Clark, Oyaert & Quin(210) studied the toxicity of urea using sheep with fistulated rumina. In sheep on hay 10–15 g. urea caused ruminal paresis, whereas 20 g. was needed to produce paresis after lucerne. The animals became normal again in 3–4 hr., though occasionally a much longer time was needed. After lethal doses of urea given to intact sheep, *post-mortem* examination revealed pulmonary congestion and oedema, haemorrhages in the heart and severe degeneration of liver and kidneys. In the studies of paresis of the rumen, there was a great increase of alkalinity and a smell of ammonia. Intravenous injection of 20 g. urea had no effect on sheep. Injection of ammonia was, however, attended by the expected fatal results. One sheep weighing 58 lb. was given 7 ml. Liquor ammoniae fortis in 700 ml. saline in three equal doses over 2 hr. At each injection it showed dyspnoea. At the third it passed into a coma but recovered in 1 hr. This treatment was carried out on each of three successive days with the same results each time. On the fourth day the sheep was killed and found to be quite normal. Urea poisoning was prevented by oral or intravenous acetic acid and made more rapidly fatal by sodium carbonate. A diet of hay plus casein made the animals more tolerant of urea over-dosage than one of hay alone. These authors were reluctant to attribute death from oral urea to the ammonia generated from it in the rumen, though in the reviewer's opinion their experiments in no way dispose of so likely an explanation. The origin of ammonia in the rumen of fistulated sheep has been further studied by McDonald(211). In the rumen, measurable increases of ammonia occurred when either casein or gelatine was being fed but not when zein was given. This effect was attributed to the water solubilities of gelatine and casein as against the relative insolubility of zein. On a diet consisting of starch, cellulose glucose, molasses, chopped straw, minerals, vitamins A and D and protein, ammonia in the rumen increased rapidly when casein was the protein, but there was no increase with zein. Non-protein, non-ammonia N behaved similarly to ammonia. These changes were taken to indicate that with zein bacterial synthesis is faster than proteolysis, whereas with casein the reverse was assumed to apply. In confirmation pure starch added to the rumen produced a rapid diminution of ammonia. Successful use of ammoniated molasses as a partial protein substitute for beef cattle(212, 213) and for dairy calves(214) is reported as also is the successful use of urea for milk production and growth in cattle(213,215). Kaishio, Higaki, Horii & Awai(216) in investigating the fate of urea in the gut reported that urea when introduced into the abomasum killed goats.

Increases of both weight and wool production were obtained by adding both potato and urea to a basal diet(217). When red palm oil and peanut oil were used instead of potatoes the effect of the urea was not so marked. Wheat gluten produced bigger increases of wool growth than urea plus potato. These results are reminiscent of those of Owen, Smith & Wright(218) on milch cows in which urea plus potato starch was found to be an effective substitute for blood meal in milk production. In Peirce's experiments urea failed to increase wool growth of sheep on a diet rich in fibre and poor in protein, whereas wheat gluten gave an increase of 35%. Potato starch plus 15 g. urea produced a 32% increase of yield, but an equivalent amount of nitrogen as wheat gluten produced a 64% increase. Clearly in Peirce's experiments protein was far superior to urea plus starch; probably much of the protein suffered succal digestion. In further experiments Peirce(219) tried the effect on wool growth of 7 g. urea in diets of increasing content of potato starch. As a potato supplement increased from 50 to 100, 150 and finally to 200 g. the wool production increased, though the effect of the initial 50 g. was not significant. Addition of vegetable oil in addition to starch had no effect, so that evidently the effect of the urea was on the utilization of the starch by rumen bacteria. In these experiments the effect of omission of the urea was, unfortunately, not studied. Williams & Moir(220) studied with six groups each of six lambs, the effects of different rations on the density of microbial symbionts in the rumen. The protozoa varied unpredictably but the rations fell into two groups: one giving counts of rumen bacteria of $41–44 \times 10^6$ per cu.mm. the other giving only $18–25 \times 10^6$. The former group appeared to supply more methionine and consisted of supplements of egg, casein or urea plus methionine to a basal diet of chaff 40, wheat 33·3, starch 8·4, molasses 6·7 together with NaCl, $CaHPO_4$ and

$CaCO_3$. The latter group consisted of supplements of subterranean clover seed, linseed meal, or urea alone. In this experiment egg gave the largest biological value and urea the smallest. McNaught, in these laboratories, prepared 461 g. dried rumen bacteria. This material(221) had a biological value of 88·3% and a digestibility of 73·2% when tested on twelve young female rats. She also has isolated from rumen contents of two fistulated cattle beasts about a pound of dried protozoa and a similar quantity of bacteria. Their biological value and digestibilities show that the protozoa probably help the host to assimilate rumen bacterial protein(222). The experimental rats used to determine the digestibilities and biological values digested the protozoal N more efficiently than the bacterial N. McNaught, Owen & Smith(221) studied the effects of metals and metal-chelating agents on rumen bacteria. The bacteria, grown *in vitro*, were remarkably tolerant to amounts of metals such as they would rarely meet in their natural habitat. Of the chelating agents, α-α-dipyridyl and 1–10 phenanthroline combined so avidly with ferrous iron that growth of the rumen bacteria was prevented. This inhibition was reversible by adding more iron, and in this way it was found that the rumen bacteria need about 2 parts per million of ferrous iron for growth *in vitro*. McNaught(223) has reported variations of the carbohydrates which can act as sources of energy for rumen bacteria incubated *in vitro*, maltose being used as a criterion. When maltose was present bacterial protein was synthesized; in its absence proteolysis predominated. Maltose was found to be replaceable by raw starch (or better still, boiled starch) inulin, raffinose, lactose, cellobiose, sucrose, D(+)-galactose, D(+)-mannose, D(−)-fructose, L(+)-arabinose, D(+)-xylose, but not by L(−)-sorbose, D(−)-arabinose, gluconic, glucuronic or glucosaccharic acids, mannitol, glucosamine, alginic acid, the sodium salts of organic acids including citrate, fumarate or succinate. β-Hydroxybutyric acid was partially used. With maltose or pentose over 90% of the carbon source was accountable to bacterial protein and polysaccharide, and to organic acids, methane and carbon dioxide. Sijpesteijn's(224) two strains of cellulolytic organism *Ruminococcus flavefaciens* already mentioned were iodophilic and resembled Hungate's yellow coccus(225).

Using their continuous transfer technique Burroughs and his colleagues have studied the use of urea by rumen micro-organisms incubated *in vitro*(226,227,228). In the first paper(226) they showed that more cellulose was digested in the presence of casein or gelatine than in their absence. In the second(227) they found that added carbohydrate (e.g. cane molasses, sucrose, starch or ground corn cobs) aided urea utilization provided it was itself digested. Small additions of these stimulated cellulose digestion but larger additions were inhibitory. Cellulose digestion and urea utilization were stimulated by addition of ashes prepared from molasses from immature clover hay or from mature timothy hay(228). Iron and phosphate stimulated cellulose digestion and iron stimulated urea utilization. Iron and phosphorus together were better than either alone and iron together with phosphorus and molasses ash were best of all(228). Lewis(229,230), in studying the metabolism of nitrate in the rumen, has confirmed earlier observations (see previous review) that it is reduced first to nitrite and then to ammonia. In the rumen if nitrite is produced fast enough to be absorbed, the methaemoglobinaemia observed by Rimington & Quin(231) in sheep in South Africa results. Lewis found(229) that 25 g. $NaNO_3$ or 10 g. $NaNO_2$ placed in the rumen, or 2 g. $NaNO_2$ injected caused the conversion of 60% of the sheep's haemoglobin to methaemoglobin. Mixed rumen micro-organisms(230) in Warburg manometer vessels rapidly produced ammonia from nitrate, nitrite or hydroxylamine and pH optima for the conversion of nitrate to ammonia were recorded. In this system formate, succinate, lactate, citrate, glucose, malate, mannitol all reduced nitrate but not so fast as did hydrogen gas. Glycerol, xylose, ethanol, acetic acid, propionic acid and butyric acid were all unable to reduce nitrate.

In view of the great volume of carbon dioxide produced by rumen fermentation it is of interest to know that carbonate is a milk precursor in the intact cow(232). The same group of workers(233), using heavy carbon, have shown that acetate with the methyl group marked appears in the lactose and the casein as well as in the fat of milk, though the biggest concentration is in the fat. With isotopic acetate Popjak, French & Folley(234) have shown the utilization of acetate for milk fat synthesis in the goat. Zelter(235) reported stimulation of milk fat production by addi-

tions of acetate or butyrate or a mixture of the two to the cow's diet. Crowcroft(236) has demonstrated that coprophagy occurs in the common shrew. Since this animal has no caecum the role of coprophagy in the shrew must differ somewhat from its role in the rabbit. Heald, Oxford & Sugden(237) have described a convenient method of preparing comparatively pure suspensions of living ciliate protozoa from the rumen. Their genera were *Isotricha* and *Dasytricha*. With such suspensions in Warburg vessels gas evolution occurred with glucose or cellobiose, but not with lactose. At the same time 'glucosan' was stored in the protozoal bodies. Galactose appeared to prolong the life of the protozoa without giving rise to any gas. El-Shazly(238), in studying the degradation of proteins and amino-acids in the sheep's rumen, found that isobutyric and the C_5 acids arose together with ammonia. This was presumed to indicate breakdown of amino-acids. Experiments with washed suspensions of rumen bacteria and amino-acid substrates confirmed this hypothesis. The partition of the fatty acids from C_2 to C_6 was determined. Clark & Quin(239), supplementing poor hay with molasses and various sources of nitrogen, found that either urea or sodium nitrate increased the consumption of the hay, as also did a supplement of lucerne hay by itself. Although the percentage digestion of cellulose was unchanged, the total digested was increased by 30%. None of the supplements enabled the sheep to maintain weight. Addition of 6% $(NH_4)_2SO_4$ or 8% of $NaNO_3$ with 12% molasses to the poor hay caused a rapid loss of appetite, which, though the authors do not say so, may have been due to a disturbed acid-base balance. With either salt at 4% depression of appetite changed to a stimulus. Neither molasses alone nor $NaNO_3$ could stimulate appetite.

Supplementary nutritive values of various concentrates when fed with various sorts of hay have been the subject of many investigations(240,241,242,243,244,245,246,247,248,249). Digestibilities of the proximate principles of various forages were compared in twenty-two steers and thirty-six wethers with increasing age of the forages as the season progressed. Duplicate cuts of the plots fed to animals in the stalls gave digestibilities by the lignin method and so enabled the intake of the grazing animals to be determined by Lancaster's method(249). The diminution of digestibility of organic matter with increase of crude fibre was at the same linear rate as its diminution with increase of lignin. Comparisons(250) of hays of different qualities showed that their proximate principles were equally well digested by either steers or lambs. Alfalfa ash(250) increased the digestibility of crude protein and crude fibre in a ration of timothy hay and a concentrate mixture. A watery extract of alfalfa had earlier been shown to increase the digestibility of a ration in which corn-cobs were the sole source of cellulose. Tarroni(251) found that dried tomato waste could replace hay for fattening cattle. Even in rations in which the digestible crude protein and total digestible nutrients were held constant the milk yields of cows reflected the differences (12–22·7%) of the crude fibre content of the rations(252). The higher the crude fibre the lower was the milk yield. Goodwin(253) finds foetal fructose in ungulates but not in carnivores or rodents. That this difference is due to phylogeny rather than nutrition is shown by the presence of fructose in the pig foetus but not in the foetus of either the guinea-pig or rabbit. Ambo & Chiba(254) found that in the horse digestibility of crude fibre (crude cellulose) was reduced by fine grinding. This probably has a bearing on the necessity of maintaining an optimal roughage intake in order to maintain the percentage of fat in the milk(255,256). Evidently particles of cellulose of too small a diameter will not remain long enough in the rumen or caecum for efficient bacterial action to occur, thus diminishing the supply of lower volatile fatty acids for synthesis into milk fat. Hope, Erb, Blosser, Ashworth & Shaw(257) found diminution of milk yields when a mixture of ground grains and peas was replaced by powdered dehydrated grass-clover mixture. Interest in the composition and chemical characterization of lignin in grass and forages continues(258, 259,260,261,262). Blaxter and his colleagues(263,264), at this Institute, have studied the nitrogenous metabolism of the young Ayrshire calf with respirometric measurements from which energy changes on various diets can be measured. A spirometer and respiration calorimeter were designed and built especially for these experiments(263). The diets were somewhat similar to those used by the workers at Illinois in experiments already quoted(265,266,267,268,269) and consisted at the outset of a reconstituted milk substitute which contained skim milk powder,

lard, glucose, mineral mixture and cod-liver oil. In later experiments the cod-liver oil was replaced by sources of vitamins A and D which were free from the toxic fatty acids of the cod-liver oil from which muscular dystrophy resulted (see section on vitamin E). For the N-free regime the skim milk was replaced by more lard, more minerals, more glucose and yeast extract. Three calves, each of which had had its dam's colostrum, were used. The endogenous urinary N was 82 mg./kg. body weight/day. Respiration calorimetry showed the basal energy metabolism to be 43 kcal./kg. body weight per day, the ratio of these two quantities being similar to that for mature animals found by earlier workers. On the N-free diet, as would be expected and as the authors point out, the output of urea plus ammonia decreased. In cattle experiments, unless almost impracticable precautions are taken, the ammonia excretion in the urine means nothing by itself, urinary ammonia being merely an index of faecal bacterial contamination (unpublished observations of the reviewer). Blaxter & Wood(270) went on to show that the metabolism of the starving young Ayrshire calf differs from that of the calf on a N-free diet. The well-known N-sparing effect of a N-free diet was observed so that the output of urea plus ammonia was much less from the calves on a N-free diet(264) than from those which were starved(270). The loss of N in the urine was 250 mg./kg. body weight/day. The extra sulphur excreted by calves starved completely as compared with those starved only of N was in the form of ionic sulphate. Ketonuria was observed only on re-alimentation. There was no evidence from the mineral output, of catabolism of bone. The heart rate fell during starvation. The respiration rate declined linearly during 4 days and bore no relation to the plane of nutrition prior to the fast. In a further experiment by the same authors(271) five calves were fed three different levels of dried skim milk made equicaloric with glucose and fed at three different planes of nutrition. On the diet with 14% protein in dry matter, the digestibilities of dry matter, fat and N tended to be lower at the lower intakes than the corresponding digestibilities of the ration with 22% protein. On the higher intakes these differences were not found. The biological value, calculated by Mitchell's method, was 65 when the protein intake was higher, and was 88 when the intake of protein was lower. This difference was attributed to the use of protein as a source of energy in the former conditions which would result in greater output of urea and a consequent lesser percentage of retention of N in the calf's tissues. Cows whole milk was fed to calves(272) at rates of either 2·6 or 6·6 l. per day. Digestibilities of dry matter, fat and protein were unaffected by the difference of intake and weight increase and storages of N, Ca and P were larger on the larger intake. Urine N did not change much with increased intake. The basal metabolism was 54 kcal./kg. body weight to the power 0·73 per day at the lower milk intake and 80 cal. at the higher. The calf with the lower metabolic rate was the less active of the two. In further experiments(273) Blaxter compared C, N and energy balances resulting from intakes of 4 or 8 l. of whole milk. The net availability of the metabolizable energy was 84·5% and the metabolic requirement per kg. to the power 0·73, body weight was 144 kcal. metabolizable energy or 122 kcal. net energy. The net energy value of the whole milk of 4% fat content and 753 gross kcal./kg. was 608. Blaxter & Wood(274) gave calves gelatine or casein as the sole source of protein. Like a non-ruminant animal the young calf was in positive N balance on the casein and in negative balance on the gelatine. Dried skim milk and casein led to weight gains but on the gelatine, weight was lost. Biological values were 92, 79 and 29·5 for the skim milk, casein and gelatine respectively. It is doubtful whether any useful meaning can be attached to a biological value such as the last 29·5 which results from a negative N balance for it can be shown that the positivity of this biological value is due to the fact that the negative balance on the gelatine diet is numerically less than the sum of the so-called endogenous N and the so-called faecal metabolic N. On Mitchell's theory(275) these last two quantities are introduced as a charge against the protein under test and are, in fact, added in his formula for the biological value, to the retained N although in fact, of course, they are not retained. It is hardly logical, however arithmetically possible, to charge a protein with a function which it is shown in the experiment to be unable to perform, since life cannot be maintained on a negative N balance. Moreover, it is obvious that any correlation between the amino-acid content and biological value of singly-fed proteins has no meaning unless each can,

in the conditions of the experiment, maintain the growing test animal in positive balance of N. If one essential amino-acid is deficient this is obviously impossible. In this connexion the following quotation from Block & Mitchell(269) is to the point: 'Supplementary relations between proteins are revealed more completely and easily by a determination of amino-acid composition than by biological estimation.'

Comberg(276) found that calves on whole milk (3·28% fat) thrived better than calves on partially skimmed milk (2·79 or 2·32% fat). Wallace, Loosli & Turk(277) found that, in general, calf-rearing formulae with much dried skimmed milk were best. Those with much whey or much fat-rich soybean meal were very unsuitable. Flipse, Huffman, Webster & Duncan(278) found that glucose plus lactose in the calf diet produced greater appetite and consequently far better growth than did either glucose plus corn syrup or glucose alone. Blood composition was unaffected by the supplements. The same workers(279) found corn syrup plus lactose superior to starch plus lactose and much superior to starch alone in stimulating growth of calves. Brumbaugh & Knodt(280) found skim milk superior to dried skim milk, red dog flour, corn gluten meal or dried whey as supplements to a basal dried skim milk diet. Sarkar, Luecke, Duncan & Ely(281) have compared the compositions of bovine colostrum and milk in respect of ten amino-acids. Colostrum was twice as rich in tryptophan as milk. Sirry & El-Sokkary(282) found that, contrary to prevailing opinion, lambs thrived well on buffalo milk. Bunge & Kamya(283) have measured the milk intake from birth to weaning of calves in East Africa. Paul & Anantakrishnan(284) report that the colostral fats of goats and sheep resemble those of cows and buffaloes in having smaller Reichert-Meissl and Polenske values, larger iodine values and larger refractive indices than those of mature milk. Reichert-Meissl values were as small as 13·2 and 14·5 in goats and sheep respectively, the corresponding iodine values being 42 and 44 respectively. These figures probably mean that colostral fat is much more a translocated depot fat than a product of acetic acid arising in the rumen. Blood fat analyses(285) showed that glycerol monostearate stimulated the absorption of hydrogenated cottonseed oil whether the oil was homogenized or not. Calves receiving the fat which had not been homogenized showed the circumanal depilation reported by Bate, Espe & Cannon(286) earlier and by De Man(287). Analyses of the contents of the alimentary canal of calves at birth has been made for proximate principles and nineteen elements by Parrish & Fontaine(288). Shanks(289) has reported scouring in calves fed on milk from cows grazing new leys. Alloxan(290), even in lethal doses, did not produce diabetes in bull calves.

Many investigations have been recently made in England into the nature of the protective action of colostrum. It would seem that the value of the vitamin A in the colostral fat has been very much over-rated and that the chief reason why colostrum is protective is its richness in antibodies specific against diseases to which the dam has been exposed(291,292). In these experiments at Reading(293,294,295,296,297) special precautions were taken to see that all experimental animals were adequately supplied with vitamins. McEwen(298), from experiments in Scotland, dissents from the view that colostrum is important on account of the specific immunities which it can confer on the calf. He produces evidence from which he concludes that the calves in his experiment were of naturally resistant types. It should be stated that in the Reading experiments Aschaffenburg(293) tested the blood of experimental calves for the presence of immune globulins to see whether or not they had suckled their dams. Aschaffenburg *et al.*(299) found no difference in resistance to deprival of colostrum between Friesian and Shorthorn calves. Eleven out of twelve of each species died from such deprival. Comline, Roberts & Titchen(300) have shown that in neonatal calves and goats (up to 27 hr. old) antibodies enter the system through the intestinal lymphatics. They used for these experiments fat-free colostral whey labelled with *Brucella abortus* agglutinin. In animals 63–65 hr. old only a slight rise of lymph titre was found. Flagellar antigen to *Salmonella dublin* gave similar results. Apart from the neonatal permeability of the gut to protein illustrated in these experiments it would seem that another natural provision for facilitating the transfer of immune globulins is the presence of antitrypsin in colostrum. In the milk of the cow whose young is so dependent on mammary transfer of passive immunity there is

more antitrypsin than in the milk of woman whose child receives passive immunity by placental transfer(301). Filling skim milk with colostral fat(302) had no effect on growth of calves in spite of the consequent large increase of intake of vitamin A of experimental animals compared with controls. Calves fed colostrum for 60 days(303) had more vitamin A and carotene in their blood plasma than controls. After the 60 days, equality of vitamin A contents soon supervened. The extra antibodies consumed did not prevent scours but seemed to aid in preventing a fatal outcome.

Williams & Knodt(304) found calf starters with 50% dried milk were consistently better than those with 20% or less of this ingredient. Soybean oil meal and corn gluten meal were of equal value. Dried corn distillers' solubles could effectively replace dried brewers' yeast. Ground raw soybeans (50% in starter) were toxic. All the animals receiving them died. Kastelic, Bentley & Phillips(305) fed colostrum-deprived calves an imitation milk made of casein, cerelose and minerals with the Ca:Na ratio adjusted to permit coagulation by rennet. This mixture was adequate provided streptomycin was given also. Butter oil emulsified into it with lecithin gave better growth than did cottonseed oil similarly dispersed. However, an excellent response occurred when cottonseed oil was emulsified in water with soya lecithin before it was added to the casein-cerelose solution. Milk coagulated with an *acidophilus* starter was believed to diminish the incidence of dysentery if it was fed for the first 10–15 days(306). A concentrate of animal protein factor (Lederle Laboratories) when used together with crystalline aureomycin increased the growth and reduced the scouring of young calves(307). In experiments by another set of workers(308) the growth rate of calves was unaltered by ingestion for 7 weeks of a concentrate of animal protein factor. Rusoff(309) found that an addition of 2% of aureomycin to the food of five ruminating calves 14 weeks old stimulated growth by 35% in the first 6 weeks of treatment. After 20 weeks the superiority over the five control calves was no longer evident, but there were no detrimental effects. Rusoff, Davis & Alford(310) added 2% of crystalline aureomycin to an all-vegetable calf-starter. Growth of Jersey and Holstein calves was 20% better than that of controls for 16 weeks from weaning. The effect was not due to vitamin B_{12} or other factor, and was attributable to an increase of appetite. Pure aureomycin prevented early scours. The effect was not explicable by any obvious changes of rumen flora. For dairy calves Knodt & Bloom(311) found that supplements of vitamin B_{12} or methionine were of no value. McKay, Riddle & Fitzsimmons(312) found aureomycin plus vitamin B_{12} to stimulate growth of calves irrespective of the presence or absence of scours in the controls. Murley, Jacobson & Allen(313) found that in young dairy calves both whole milk and reconstituted skim milk gave greater live-weight gains when aureomycin was fed as well. The total digestible nutrients per lb. live-weight gain was unaltered. All the calves received alfalfa hay *ad lib.* plus less than 4 lb. of a mixture of ground corn (40%), crushed oats (30%), soybean oil meal (28%), steamed bone flour (1%) and salt (1%).

WATER SOLUBLE VITAMINS IN THE NUTRITION OF THE CALF

Before the development of the characteristic flora and fauna in the rumen, young ruminants are dependent on exogenous supplies of vitamins of the B-complex which they obtain from colostrum and milk. Before 1948(314) biotin deficiency had been shown to produce a paralysis of the hind-legs of the young calf, and deficiencies of thiamine, of riboflavine and of pantothenic acid had been experimentally induced. Kesler & Knodt(315,316), in calves from 2 to 16 weeks of age, found at slaughter several times as much riboflavine and nicotinic acid in all parts of the gut contents as there was in the food. There did not in these observations appear to be any synthesis of thiamine. These differences were not affected by the time between the last feed and slaughter(316). Teeri, Josselyn, Colovos & Keener(317) found that hay which was poor in thiamine, riboflavine and nicotinic acid led to decreased excretion of these factors in urine and faeces. Workers in Illinois, U.S.A., using diets for calves and lambs of casein, lard, minerals, cerelose, vitamins A, D, E and K and nine factors of the B complex have studied the effects of omission of some B complex factors. In this way deficiencies of riboflavine(266) and pyridoxine(267) have been experimentally induced in calves. With calves on such a diet, but with increasing amounts of added riboflavine,

it was shown that judging by both maintenance of body weight and excretion of riboflavine in the urine the calf's requirement of riboflavine was about 6 mg./100 lb. body weight per day. This value may be compared with the 1–4 mg. riboflavine necessary for the pig in which Mitchell *et al.*(268) found an increase of the requirement with increase of environmental temperature. In the calf(267) a premortal symptom of pyridoxine deficiency was the onset of epileptiform fits and at examination *post-mortem* demyelination of nerves was found. In deficient calves urinary excretion of pyridoxine and its metabolites was reduced. Onset of symptoms could be prevented by pyridoxine, by pyridoxal or by pyridoxamine. The same workers(269) have reared newborn lambs on a diet similar to that used for the calves but free from pteroylglutamic acid. Growth was not impaired, but a leucopaenia curable by injection of folic acid was produced by the diet. The leucopaenia could also be produced by the 'anti-metabolites' aminopterin or methyl folic acid, but calves were only temporarily leucopaenic after aminopterin to which they seemed to become adapted. Moody, Hauge & Lundquist(318) report that by ultra-violet irradiation at 60–65° F. the riboflavine in milk can be destroyed without changing its flavour, and Brisson & Sutton(319) have used such milk to estimate the riboflavine requirement of the newborn calf. The calves had their dam's colostrum for 48 hr. and then the experimental diet for 8 weeks. The milk was supplemented with vitamin A which was for the most part removed from the original milk during the ultra-violet irradiation. The requirement found in the experiments was 1·6–2 mg./100 lb. body weight. This is considerably less than the minimal requirement quoted earlier as found by Draper & Johnson(266) of 6 mg./100 lb. body weight. However, in all these experiments the excretion of riboflavine in the urine was measurable even when the calves were on the deficient diets and the diet of Draper & Johnson was much more artificial than that of Brisson & Sutton. Moreover, Brisson & Sutton found that at the end of the experiments even the rumen contents of the deprived calves could synthesize riboflavine on incubation *in vitro*. In attempting to estimate a calf's requirement of any B-complex vitamin one may well be firing at a moving target. If a partial deficiency is being estimated, agreement as to results is hardly to be expected. Luecke, Culik, Thorp, Blakeslee & Nelson(320) have induced riboflavine deficiency in lambs on diets very similar to that used by Draper & Johnson(266). In these experiments ten factors of the B-complex were added, including choline and pteroylglutamic acid. In the blood there was no evidence of anaemia or of leucopaenia resulting from the deficiency. Chanda & Owen(321) have confirmed earlier work from Kon and his colleagues at Reading, that in colostrum as compared with milk a lesser proportion of the thiamine is present as cocarboxylase. Baldissera(322) studied the synthesis of thiamine in the rumen of two goats to which he administered various sulphonamide drugs, one of which *p*-aminobenzene-sulphonamido-methyl-thiazole caused a marked rise of thiamine in the rumen. Attempts to show the synthesis of thiamine from the thiazole fragment of this drug by incubation *in vitro* did not succeed. Teeri, Leavitt, Josselyn, Colovos & Keener(323) measured the urinary and faecal outputs of thiamine, pantothenic acid, nicotinic acid and riboflavine in ruminants. For both thiamine and pantothenic acid output exceeded intake so that synthesis must have occurred. Furthermore, output in the urine equalled intake for thiamine and was greater than intake for pantothenic acid thus providing evidence for the benefit of the synthesis to the host. The output of all four vitamins was decreased by sulphathalidine administration. Reindeer(324) have more vitamin C in their milk than do cows.

TRANSFORMATION OF CAROTENE INTO VITAMIN A

Both carotene and vitamin A occur in the liver of the cow which secretes carotene in its milk fat and in the liver of the goat(325) which does not. These observations are in apparent support of the theory that carotene is transformed into vitamin A in the liver. For many years this theory was believed in spite of unsuccessful attempts(326) to demonstrate *in vivo* this alleged function of the liver and in spite of the unsatisfactory nature of the evidence in support of positive claims in this regard. As mentioned in the previous review in this series the theory of the preponderant role of the liver in producing vitamin A from carotene(327) is now generally disbelieved(75). Irrefutable evidence has been adduced by various authors that in the rat(328,329), the pig(330), the

cow(75), the sheep(331,332), the goat(75), the guinea-pig(333) and the chick(334,335) the main site of conversion of carotene to vitamin A is the intestinal wall, and McGillivray(331), Klosterman, Bolin & Light(332), and Stallcup & Herman(336) have shown that sheep intestine can convert carotene into vitamin A during incubation *in vitro*. A recent critical discussion of this transformation may be found in the recent book on carotenoids by Goodwin(326). Goodwin quotes early workers on carotene who claimed that intravenous injection of aqueous homogenates of β-carotene could be used by vitamin A-deficient rats, and recent workers have adduced evidence that the intestine cannot be the sole site of conversion though it may be the chief one. There may also be species differences involved. Certain investigators, notable among whom is Bieri(337,338), have shown that an oily solution of carotene, unlike a watery homogenate, is of no use to vitamin A-deficient rats or calves when injected. Evidence for the alternative site of conversion is, however, not convincing, and there is some evidence from Bieri(339) that the homogenizing agent may play a part, for solutions of carotene in tween 80 are not quite stable and the nature of the breakdown products still awaits proper investigation. Of interest, however, is the fact that Bieri's homogenates of β-carotene were vitamin A-active when injected intramuscularly(338). It might be thought that injected carotene could reach the intestine through circulation in the bile, but most investigators seem to agree that the bile is free from carotenoids. It is of interest to note that all the animals mentioned in this section are either herbivores or mixed feeders, for Kowalewski & Henrotin(340) and Kowalewski, Henrotin & van Geertruyden(341) have studied the conversion in the dog with results which seem to show that the dog is exceptional. They found increases in hepatic, venous and in peripheral blood after injection of aqueous carotene into the portal vein. More surprising still, when β-carotene, either aqueous or oily, was administered into the intestine it caused increases in the vitamin A in the portal blood, in the blood of the vena cava inferior and in the liver. There was no evidence of any increase in the vitamin A in the lymph taken from the thoracic duct at the same time. This lack of change of lymph composition is in complete contrast to the major function of lymphatic absorption in the ruminant(331), rat(329,330), pig(330) and guinea-pig(333). The experiments of Meng(342) on the absorption of fats by the portal route are of interest in this connexion. By comparing oral and intravenous administration of an aqueous suspension of carotene to calves depleted of vitamin A, Eaton, Matterson, Decker, Helmboldt & Jungherr(343) claimed to show on the basis of blood analysis that conversion of carotene to vitamin A occurred in some other site than the intestine. Most workers have found that the actual intestinal site of conversion is the wall. McGillivrary(344) thought caecal symbionts which he plated on agar could effect the conversion. Bieri(338) found conversion into vitamin A after injecting carotene into eviscerated rats.

Various 'tweens', e.g. sorbitan monolaurate, polyoxythylene sorbitan mono laurate, poly-oxyethylene monolaurate and poly-oxyethylene monostearate were lethal as 25% of the diet, of hamsters(345) or rats(346).

Clinicians have long suspected a connexion between carotene metabolism, toxic goitre and myxoedema(347), and the effect of the thyroid on the storage from dietary carotene of vitamin A in the liver of rats has been demonstrated(326). Chanda *et al.*(50), in these laboratories, have found that in both the cow and the goat the digestibility of carotene is increased by thyroxine and reduced by thiouracil and that the composition of the milk in the treated animals changes in the expected fashion(348,325) for thyroxine increases while thiouracil decreases the ratio of vitamin A to β-carotene in the milk. In these experiments it was found that thyroxine had a different effect on the milk when the diet contained carotene than when it did not. Thyroxine treatment on a diet containing carotene increased the ester form of vitamin A in the milk. Whenever carotene was omitted from the diet the percentage of esterification of the vitamin A in the milk was decreased and this decrease was greatly enhanced by treatment with thyroxine but was unaffected by thiouracil. The authors interpreted these results to mean that thyroxine can accelerate the change of dietary carotene into vitamin A ester in the blood and that when there is no carotene in the diet, thyroxine can accelerate the release of liver reserves of vitamin A alcohol into the blood and their transfer to the milk. The authors have produced blood analyses(349) in support

of these explanations. It had been claimed that thyroxine could convert carotene to vitamin A *in vitro*, but neither Lowry & Lowry(350) nor McGillivray(351) could confirm these claims. In their experiments on dogs Kowalewski, Henrotin & van Geertruyden(352) found that thyroidectomy prevented the power of the dogs to form vitamin A from carotene injected into the portal vein as a colloidal solution in isotonic glucose in spite of general increases in carotene in the venous blood. Treatment with thyroxine(353) restored the power of conversion. Paradoxically Bieri(338) found that rats rendered hypothyroid by thiouracil added to an A-deficient diet could be cured of xerophthalmia by 200 μg. β-carotene in a solution of tween 80 in an equal volume of water given weekly for 5 weeks either by mouth or by intramuscular injection. Tween by itself had no effect.

Patel, Mehl & Deuel(354) have found that cryptoxanthin, when given by mouth, produced vitamin A in the liver of the rat. After intraperitoneal injection it could be found in the liver but did not give rise to vitamin A. Zeaxanthin had no vitamin A-producing power even by mouth.

Many observations have been published on the composition of butter and on the well-known seasonal variation of the vitamin A and carotenoid content depending on the availability to the cows of fresh greenstuff in quantity in U.S.A.(355), in India(356) and in Azerbaijan(357). The well-known dependence of milk vitamin A on intake of greenstuff has been demonstrated in buffaloes(358,359) and in cows(360) as also has the diminishing return of vitamin A in milk with increasing carotene intake(361). This diminishing return has also been shown experimentally by comparing blood carotene, blood vitamin A ester and carotene intake by Chanda & Owen(349). Patel & Ray(362) have found that large amounts of unextracted cotton-seed depress the rate of transfer of carotene to the milk and also the rate of its transformation into vitamin A. Goodwin & Wilson(363) have confirmed the observation that β-carotene and vitamin A are minimal in the blood at or just after parturition. They attribute this to changes of blood concentration. Kosterman, Bolin & Ford(364) found that supplementation with vitamin A had no effect on the vitamin A content of the blood of beef cattle on a carotene-deficient diet. Neither was there any measurable improvement on calf production. In spite of the repeated demonstration of the effect of carotene intake on vitamin A in butter fat Stewart, McCallum & Watts(365) found that the reserves of vitamin A in the liver of cattle were out of step with the seasonal intake in three successive seasons. The vitamin A content of milk does not therefore reflect liver stores of it, and from the work of Chanda & Owen(349) there would seem to be no reason to suppose that they should. Eveleth, Bolin & Goldsby(366) have shown that symptoms of avitaminosis-A in sheep produce after 160–190 days similar symptoms of muscular inco-ordination and night-blindness to those already reported in cattle in spite of good maintenance of weight, fatness and wool production. Deficient sheep had very low reserves of A in the liver, low figures for vitamin A in the blood plasma and high cerebro-spinal fluid pressures. Blood vitamin A responded quickly to oral administration of carotene, but this response was entirely prevented by thiourea. Blood proteins were unaffected by the deficiency. Attempts to generate the deficiency in newborn lambs failed owing to death of the animals from intercurrent infections after 3–4 months. The livers of newborn lambs contained $\frac{1}{2}$–1 i.u. vitamin A/g. Lindley, Brugman, Cunha & Warwick(367) and Sapsford(368) have studied the effect of carotene deprivation on spermatogenesis in rams, the latter finding that spermatogenesis was adversely affected whether the plane of nutrition on the carotene-deficient diet was high or low. The former workers found that neither testosterone propionate nor pregnant mare serum could reverse or prevent the adverse effects of carotene-deficiency on spermatogenesis. Many of the deficient lambs had cystic pituitaries. Many workers have studied vitamin A deficiency in calves(369,370,371,372,373,374). Eaton, Helmbolt, Jungherr, Carpenter & Moore(369) found that calves lost appetite when the blood vitamin A fell to 4 μg./100 ml. Liver stores were depleted and cerebro-spinal fluid pressures increased. Exophthalmus, muscular inco-ordination and diarrhoea were other symptoms of the extreme deficiency. Thomas & Moore(370) found that vitamin A in the plasma was not a reliable index of liver storage, but in the view of the present author(349) this finding is not unexpected. Eaton *et al.*(371,375) found that artificially dried and pelleted hay maintained liver stores better than

artificially ground hay, which in turn was better than field-cured hay. Dolge *et al.*(376) studied the performance of dairy calves on standard ration to which 25, 50 or 75% of artificially dried alfalfa meal had been added. The 50% ration gave the greatest concentrations of vitamin A and carotene in the blood plasma in spite of the much bigger intake of carotene on the 75% ration. The gain of live weight was biggest on the 50% ration also, but, in spite of this, the 25% ration gave the greatest rate of increase of live weight. Obviously the 75% ration contained too much crude fibre. The absorption of vitamin A from the gut and its release from the liver(372,377) are affected by the fat content of the diet. A low fat diet(372), even though carotene-deficient, can temporarily increase the blood-vitamin A due to release of stores in the liver. Thirty per cent of ground soybeans in the diet(377) depressed vitamin A storage in the livers of calves. Crowley & Allen(378) compared corn oil, soybean oil, cow milk butter, goat milk butter and mineral oil as vehicles for dietary carotene. Mineral oil was by far the worst vehicle, and absorption from all the oils, including the mineral oil, was greatly increased by homogenization. Inoculation of the rumen of calves with cud from adult animals had no effect on carotene absorption(379). Eaton, Spielman, Johnson & Matterson(380) and Nezvesky *et al.*(381) have shown that massive doses of vitamin A given to cows continuously for some weeks before calving increase the amount of vitamin A in the livers of their calves. Ronning & Knodt(382) have shown that sulphonamide drugs have no effect on the blood vitamin A of dairy calves. Allen, Wise & Jacobson(374) have found that vitamin A cannot be detected in the blood of milk-fed calves by the glycerol-dichlorohydrin method unless the fat is a saponified. Jacobson, Wise, Allen & Kemphorne(383) report that homogenates of carotene in milk were absorbed faster when fed from a bottle than when fed by stomach tube. Gelatine capsules containing solutions in oil were absorbed more slowly still. Jacquet & Raust(384) found that meat with yellow fat has a larger content of carotenoids and vitamin A than meat with lighter fat. The stability of carotene and vitamin A in mixed feeds is still being investigated. Francois(385) reports a negative correlation between the carotene and fibre contents of lucerne hay. Thomson, Bickoff & Maclay(386) have shown that isomerization of carotene occurs during its dehydration, half being all trans-β-carotene, about one-third neo-β-carotene-B and the remainder neo-β-carotene-U. Stoerck, Kaunitz & Slanetz (with Johnson)(387) find that protracted vitamin A deficiency in the rat can be prevented by the addition of 5% of lard to the diet and posit a 'lard factor' in explanation. Hansel, McEntee & Olafson(388) found a factor in a processed wheat concentrate which causes hyper-keratosis in calves and simultaneously lowers the plasma vitamin A. McGillivray(389), using three pairs of identical twins at pasture, found that 1 g. of alfa-tocopherol per day to one of each pair increased both the vitamin A and vitamin E content of the milk. Three grams with or without 300 mg. of carotene in oil caused no further difference. Chanda *et al.*(51) have demonstrated that in goats and cows chromium oxide may be used as a marker in the feed to measure the digestibility of carotene. The concentration of total vitamin A in the blood of mature cattle cannot be readily correlated with intake of carotene or of vitamin A. This is apparently because only the ester form of vitamin A in the blood reflects the carotene intake, as Chanda(390) in these laboratories showed for grazing animals and as Chanda *et al.* showed in further experiments. When these cows were receiving a diet containing neither carotene nor vitamin A, no vitamin A ester was detectable in the blood(349). Evidence of combination of vitamin A alcohol and of β-carotene with plasma proteins has been adduced by Ganguly, Krinsky, Mehl & Deuel(391).

In determining the carotenoids and vitamin A in the colostrum and milk of the cow and the goat, Chanda(390) found vitamin A alcohol in cow's milk and in goat's colostrum but not in goat's milk. Though most of the vitamin A in cattle blood was in the form of the alcohol, and though vitamin A alcohol in the blood of the cow tended to be constant the ester varied from cow to cow and from time to time in the same cow. Chanda & Owen(349) and Chanda, Clapham & Owen(392) found that as the intake of carotene, in the form of dried grass meal, increased, the vitamin A ester in the blood increased but at a diminishing rate. Two other aspects of vitamin A metabolism deserve a passing mention. One is the preparation of rhodopsin from the retinae of bovine eyes by Collins, Love & Morton(393). The other is the discovery by Meunier, Ferrando,

Jouanneteau & Thomas(394) and Meunier, Ferrando & Perrot-Thomas(395) that vitamin A participates in some as yet unexplained way in detoxication processes. Both the formation of hippuric acid from benzoic and the formation of mercapturic acid from bromobenzene have been shown to be potentiated by its presence in the diet.

Van Arsdell, Ross & MacVicar(396) fed cows rations which produced intakes of 38, 75 and 105 mg./head of β-carotene. There was a correlation between the plasma carotene and carotene intake, but not between plasma vitamin A and carotene intake. This is not surprising in view of the results of Chanda *et al.*(392) that vitamin A ester but not vitamin A alcohol reflects carotene intake and that blood vitamin A is chiefly in the alcohol form(397).

VITAMIN E

From recent reviews on vitamin E, the role of this vitamin in ruminant nutrition still requires elucidation(398, 399, 400). Brown(401, 402) has shown that the various forms of vitamin E—α, β, and γ—tocopherol can be separated by reversed phase chromatography on filter-paper and has shown great variations in the proportion of the various forms in various vegetable oils. Barnicoat(403) found that in New Zealand the carotene and vitamin A were minimal in midsummer. This is reminiscent of the observations of Stewart *et al.*(365) on the minimal hepatic vitamin A at midsummer, in Scotland. McGillivray found that the minimum in butterfat was independent of the date of calving. Using three pairs of identical twins McGillivray(127) found that the minimum is probably due to a lower content of vitamin E in midsummer pastures for the potency in vitamin A could be restored by dosing one of each pair of twins with vitamin E, a treatment which markedly increased both the vitamin A and the carotene in the milk. A significant positive correlation was found between the contents of the two vitamins in the milk. Supplementation with carotene in oil was ineffective in increasing the vitamin A in the milk. Krukovsky & Loosli(404) found that the milk from cows on standard winter rations of late-cut timothy hay, corn silage and concentrates gradually became poorer in vitamin E as the winter drew on and that proneness to oxidized flavour induced by copper increased. Supplements of 1–2 g. per day of mixed tocopherols improved the milk if given early enough in the season. Parrish, Wise, Latschar & Hughes(405) found that prepartal supplements of tocopherols did not very much increase the serum tocopherols in calves at birth, but that the ingestion of colostrum caused a large increase of serum tocopherol which was larger still in calves of supplemented dams. After 28 days no difference between the calves of the supplemented and unsupplemented dams was distinguishable. Kachmar, Boyer, Gullickson, Liebe & Porter(406) found that vitamin E supplementation had no effect on the vitamin A or carotene in blood plasma of cows. Piana(407) found that rancid fats produce testicular lesions in male calves. Vawter(408) produced recovery in subacute cases of 'white-muscle disease' of calves by injections of mixed tocopherols. In the acute form the disease is characterized by stiffness of the legs, prostration and inability to nurse. A similar treatment cured 'stiff lamb' disease(408). For a long time it has been known that cod-liver oil can produce toxic symptoms not attributable to excess of vitamin A or vitamin D and a form of nutritional muscular dystrophy has been produced in several animal species. Nevertheless, in the experiment of Culik, Bacigalupo, Thorp, Luecke & Nelson(409) lesions in skeletal and cardiac muscles of lambs were produced by a diet deficient in vitamin E and cod-liver oil did not increase the rate of appearance of symptoms in lambs on the deficient diet. Blaxter *et al.*(410, 411, 412) have produced muscular dystrophy in calves on diets containing cod-liver oil. In these experiments 50 mg. vitamin E could not completely antagonize 15–18 ml. cod-liver oil. Tri-*o*-cresyl phosphate is a contaminant of oil of parsley seeds which has been used as an abortifacient. Certain workers in Germany, during the recent war, used tri-*o*-cresyl-phosphate for frying fish when fat was scarce. They developed the well-known polyneuritic symptoms(413). It is interesting, therefore, to hear from Draper, James & Johnson(414) that paralysis of the hind-legs of lambs resulting from diet poor in vitamin E is aggravated by the addition of tri-*o*-cresyl phosphate to the diet. In these experiments lambs receiving both vitamin E and tri-*o*-cresyl phosphate did not develop the dystrophy. These effects and the fact that in chicks E-deficiency causes ence-

phalomalacia make one wonder whether the primary lesion of nutritional muscular dystrophy is nervous rather than muscular. Lesions of the heart muscle are a frequent cause of death in deficiency of vitamin E in cattle. Of twenty-eight animals which were kept on rations poor in vitamin E(415) for 1 year, thirteen died of heart failure at ages varying from 21 months to 5 years. The breeding performances of both male and female animals had been very good. Autopsy revealed slight brain, bowel, heart and pancreatic haemorrhages. These experiments may have been complicated by a lack of vitamin K in the E-deficient ration.

CALCIUM, PHOSPHORUS AND VITAMIN D

Aphosphorosis is still a hazard of dairy cattle. In Ireland(416,417,418,419) it causes lameness known as 'bog-crook', the incidence of which can be obviated by supplementary feeding of calcium phosphate or bone-flour, or better by fertilizing pastures with superphosphate(420). It has been reported from cattle in East Anglia in England(420) and from sheep in Utah in the U.S.A.(421). The English cases were attributable to insufficiency of natural grazing and the American ones to inadequacy of winter feeding. Hallsworth & Clowes(422), in a study in New South Wales of the relation of pasture to milk production, concluded that the phosphorus intake from grass could be a natural limitation of milk production. Hignett & Hignett(423,424) go so far as to say that subclinical aphosphorosis is a cause of poor breeding performance in cattle in some parts of England. They assert that the Ca/P ratio of grazing is far from optimal (compare reference(22)). Abrams(425) very strongly discounts the conclusions of Hignett & Hignett. Kivimäe(426), in Finland, asserts that pasture alone cannot supply the mineral requirements of cows. The effects of treatment with excessive amounts of P have been studied by Lewis, Burkitt, Seghetti & Willson(427) and by Saarinen(428). Thomas(429) has correlated delayed closure of the ulna epiphyses with bone-ash, Ca, P and phosphatase analyses in seventy-five calves, and Benzie(430) has charted by X-rays the growth of the skeleton of the foetal sheep. Strobino & Farr(431) have studied gradients in the distribution of N and ash in bovine and human bones. The radius, ulna and rib showed medial maxima of ash and medial minima of N content. This probably means that these bones are strongest per unit weight where they are thinnest. Rogers, Weidmann & Parkinson(432), in a study of bovine, human and rabbit bones, find that the Ca/N ratio is attributable to true differences of calcification. Of the bone protein 90–96% is collagen or collagen-like proteins. The present reviewer(433) has recently discussed bone as a mineral reserve. Gallup & Briggs(434) found that rations containing 30% of their N as urea did not affect the Ca and P balances of lambs. The requirement of P was 2 g./100 lb. live-weight per day. Saarinen, Comar, Marshall & Davis(435), by studies with radiophosphorus, concluded that casein P and serum P came from acid-soluble P in the blood plasma. The same laboratory(436) reports that ^{45}Ca when injected into cattle is rapidly spread throughout the skeleton and all parts of the alimentary tract. Active secretion of Ca into the bovine gut was also shown by the present reviewer(437). Radiophosphorus has been used to measure metabolic faecal P directly in cows(438). In one such experiment the apparent digestibility of P was 12%. When this was corrected for the metabolic faecal P of 14 g. per day the true digestibility became 50%. In this experiment there was 43% of endogenous P in the faecal P. In another such experiment in which the apparent digestibility was negative, the true digestibility was 64% and the endogenous P of 10 g. weight was 70% of the faecal P. In these experiments no attempt was made to measure rates of re-absorption after ruminal or rectal injection. In similar experiments(439) with calves apparent digestibilities of 74 and 75% were found to represent real ones of 93 and 94% respectively. Phosphorus excretion into the gut was shown to occur all along it but especially into the jejunum(440). Aten & Heyn(441) injected radio-calcium into a lactating goat. The activity of the milk Ca became equal to that of the blood plasma after 4 hr. Simmonnet & Sternberg(442) found that in the mammary gland of the bitch and the rat the greatest specific activity from intravenously injected ^{32}P phosphate was in the inorganic fraction. They posited phosphopyruvic acid as a precursor of phosphoserine but produced no chemical evidence for this hypothesis. From the work of Malpress and his colleagues(443) glucose-phosphate would seem a more likely

precursor for they have accounted for the major part of the ester phosphorus of milk in this form, and Chanda & Owen (444) have shown a very large increase in this fraction in milk after thyroxine treatment and at the peak of lactation. Casein containing ^{32}P when fed to a young calf provided no evidence of absorption of P as either phosphopeptone or phosphoserine (445).

VITAMIN D

Green (446) has made a study of chemical methods of estimating vitamin D. The chemistry of vitamin D has also been studied by Garkina & Bukin (447) and by Davis, McMahon & Kalnitsky (448). Sneider, Eisner & Steenbock (449) have determined vitamin D activity from its effect on the disintegrations per minute, in the forepaw of the rat, of injected ^{32}P. By means of a diet of dried beet pulp, chaffed oat straw, blood-meal, urea, molasses, calcium carbonate and salts Ewer (450) produced rickets in young sheep. The P in the diet (0·08% or 0·3 g. P per sheep per day) was about one-seventh of the optimum. The control diet, rich in P, had 1·2% of this element. This level of P prevented rickets but with vitamin D_2 in addition gave the best results. In these experiments a Ca/P of 2·9/1 produced rickets. A ratio of 14/1 produced hypophosphataemia without rickets. The rachitic sheep (451) were in negative P balance. Vitamin D_2 reduced the negativity of the P balances and made some of them positive. Hibbs, Pounden & Krauss (452), in studying milk fever, treated cows with vitamin D ante-partum but failed to reduce the incidence of milk fever, although both Ca and P in the blood plasma were increased. Magnesium was unaffected by the treatment with vitamin D. Vitamin D in the blood was 10 times as much as was present in the blood of control cows. In spite of persistent experimentation the metabolic role of vitamin D still lacks precise description. Carlsson (453), from experiments with ^{45}Ca believes its functions to be homeostatic for he found that, on diets very deficient in Ca, it facilitated the removal of Ca from the bones. In the same experiments he found no evidence of return of ^{45}Ca once it had been deposited in the teeth. Harrison & Harrison (454), using ^{45}Ca, found that Ca was absorbed mostly from first two-thirds of the small intestine, and that absorption from the distal third occurred only in one of two sets of circumstances, namely during the feeding of Ca after a period of Ca deprivation, or during the administration of vitamin D. Underwood, Fisch & Hodge (455), also using ^{45}Ca, confirmed earlier observations that vitamin D increases absorption from the gut and facilitates the mineralization of bone. Manunta & Mureddu (456) found that vitamin D_2 had no effect on Ca in the milk of the ewe, though parathormone caused milk Ca to increase. Recently the known effects of certain organic acids in favouring mineralization of the skeleton (453) have received further attention. Steenbock, Bellin & Weist (457) found that vitamin D increased the acidity of the contents of the caecum and the colon. Bellin & Steenbock (458) reported that, in either physiological or massive amounts, vitamin D increased citraturia in young or adult rats, kept on a rachitic diet poor in P and containing $NaHCO_3$. Citrate output in the urine was considerably reduced by adding 0·54% of NH_4Cl to the diet when vitamin D was not given. Even when vitamin D was given its effect of increasing citraturia could be completely nullified by 1·07% NH_4Cl in the diet. Evidently if a ration contains, as did those of Steenbock and his colleagues, an excess of Ca, this excess can be eliminated only by a fluid in which it is soluble, in this case an acid urine. Ammonium chloride will produce such an acid urine, and hence will obviate the necessity for the operation of the citrate mechanism. In these experiments urinary citrate increased when either the Ca in the diet or the alkalinity of its ash was increased and vitamin D enhanced both effects. Taüfel & Krusen (459) found citric acid to be an essential constituent of the bones of eight species of animals, including man and ox. Arvy & Gabe (460) assert that thyroxine has a vitamin D-like action and perhaps, in view of the known effect of the hormone on phosphorylations, this is not surprising. Evidence has been adduced that phosphorylated vitamin D_2 activates alkaline phosphatases of kidney, intestine and bone (461, 462). Theopold (463) has investigated the distribution of alkaline phosphatase activity in various tissues of normal and rachitic rats. In the latter phosphatase in intestines, kidneys and tibiae was bigger than in normally fed rats. Vitamin D caused the values to approach normality while citric acid caused some improvement. In the skeleton, phosphatase activity was zero in cartilage

and greatest in the periosteum, other skeletal tissue falling in between these two extremes. Grant(464), in studying the rachitogenic effect on lambs of young green cereals(465), has prepared from oats and from Italian rye grass a chloroform extract which has anti-vitamin D activity. Newlander & Riddle(466) have again shown the importance of insolation for generating vitamin D-activity in hay, and Thomas & Moore(467) have shown that the vitamin D activity of alfalfa hay increases with increase in the number of dead leaves on the plants at harvest.

COBALT AND VITAMIN B_{12}

Since the last review(327) in this series the role of Co in ruminant nutrition has been considerably elucidated. The present state of knowledge of the role of the trace elements Co, Cu and Mo in plant and animal biochemistry has been ably summarized by Marston(468). The occurrence and correction of Co pine in the field has been discussed by Smith(469) and Lee(470) in Australia and by Cunningham(471) in New Zealand. Cobalt deficiency in sheep on farms in Old Red Sandstone areas of Herefordshire and Worcestershire has been reported by Osborne, Patterson & Treanor(472). Smith, Becker, Loosli & Beeson(473) have described Co deficiency of sheep in New York State, and Vsyakikh(474) has described the experimental deficiency in sheep in the Soviet Union. In Vsyakikh's experiments the distribution of Co in the tissues of deficient and Co-supplemented animals was studied. Co in the pituitary rose from 89 μg. % at birth to 863 μg. % at 6 months. In experimental ewes the pituitary, thymus, pancreas, thyroid, lungs and spleen contained 246, 4, 8, 9, 4 and 0 μg. % respectively. The corresponding figures for Co supplemented ewes were 863, 29, 30, 40, 27 and 17 respectively. Vsyakikh recommended a dose per week, varying with age and sex from 10 to 25 mg. $CoCl_2$. Stewart(475) has further described his method of producing Co deficiency in lambs. He used hay from an area where Co deficiency was known to occur. Ewes were fed this hay together with flaked maize for the last 3 months of pregnancy. The intake of Co was 69 μg. per day. Lambs were weaned at 10 weeks and then kept on the above diet. They started to lose weight at 14 weeks but started gaining weight if given Co by mouth. Lambs not so supplemented died. Phillipson & Mitchell(476) found that lambs in which the deficiency had been similarly produced could be cured by 100 μg. Co per day by mouth. A similar dose given either intravenously or into the duodenum was ineffective, but larger doses into the duodenum or abomasum were effective. These larger doses reached the rumen presumably by retroperistaltic movements of the gut. Co, whether administered by mouth or intravenously, found its way to the liver but liver reserves were of no diagnostic significance. Das & Stewart kept lambs in a Co-deficient field(477) which they divided into two by a central fence. One half of the field was manured with Co and superphosphate, the other with superphosphate alone. The Co led to increased appetites and live-weight gains. Keener, Percival, Ellis & Beeson(478), contrary to earlier observers, report that injected Co sulphate if given daily or in larger doses twice weekly will cure Co pine. In these experiments B-vitamins, liver extract, methionine, tryptophan, a Co-free salt mixture, fresh rumen liquid and fresh raw milk were ineffective. This is probably explicable by experiments with ^{60}Co of Monroe, Sauberlich, Comar & Hood(479), who showed that the conversion of Co to vitamin B_{12} was 10 times as much after oral as after intravenous administration. It is now generally agreed that the function of Co in the ruminant is to enable symbiotic rumen bacteria to make the various forms of vitamin B_{12} from it and so to facilitate normal blood formation in the host(468,480,481,482,483,484,485,486). There are large quantities of vitamin B_{12} in sheep and calf faeces(487,488) and in rumen contents(487,489,490). A satisfactory explanation is still lacking as to why the ruminant has such a large requirement of dietary Co. Ford, Kon & Porter(488,489), who investigated the vitamin B_{12} like factors in calf rumen contents and in livers of cattle, found four such factors in faeces and rumen contents but only vitamin B_{12} in the livers. They suggest that Co has a dual function in ruminants: first to supply the rumen bacteria with essential growth factors and secondly to supply vitamin B_{12} to the host. Hale, Pope, Phillips & Bohstedt(487), by feeding chicks rumen contents or faeces of sheep, found more vitamin B_{12} in the materials from Co supplemented than from deficient sheep. Dawbarn, Hine & Hughes(484) found that the ratio of *coli* to *leichmanii* factors in sheep faeces

was markedly increased by oral Co. Injected vitamin B had no such effect. In the urine both factors were increased by oral Co. Considering true vitamin B_{12} to be indicated by the alkali-labile portion of *Lactobacillus leichmannii* 3797 activity, Hoekstra, Pope & Phillips(480) found that Co-supplemented sheep had more B_{12} in the liver than did pair-fed animals on a Co-deficient diet. Total B_{12} in rumen contents and blood was also increased but Co had no effect on the nicotinic acid or riboflavine in rumen contents. All the pair-fed animals lost weight because of the poor appetities of the animals deprived of Co. Nevertheless, comparable animals fed *ad lib.* showed the same changes of vitamin B_{12} in blood, rumen contents and liver. Hale, Pope, Phillips & Bohstedt(490) report that the anaemia of Co deficiency is amenable to treatment with a mixture of B-complex vitamins containing folic acid and vitamin B_6 but not vitamin B_{12}. These authors therefore suggest a role of Co other than its power to generate vitamin B_{12}. Koch & Smith(491) found vitamin B_{12}, vitamin B_{12b} or Co chloride equally effective in alleviating Co deficiency in lambs. Anthony, Rupel & Couch(492) found 0·72–1·19 mg. vitamin B_{12} per ml. blood of cattle eating grain, alfalfa hay, silage and pasture. Harper, Richard & Collins(493) found a highly significant increase of vitamin B_{12} in ewes' milk after feeding extra Co. Becker & Smith(494) alleviated Co pining by injecting lambs with liver extract. Keener, Baldwin & Percival(495) found evidence of passage of injected ^{60}Co into the rumen. This probably accounts for the curative effects reported by some workers who gave Co injections. The route by which Co reached the rumen was not investigated. Draper *et al.*(265) unsuccessfully attempted to produce a vitamin B_{12} deficiency in the calf with a diet which in pigs caused a growth inhibition reversible by vitamin B_{12}. Their diet contained Co in the salt mixture. Excessive doses of Co are toxic to sheep(496) and to calves(497). To a calf a dose of more than 4 mg./lb. body weight is lethal. Toxic symptoms produced by doses greater than 0·3 mg Co per lb. body weight are lacrymation, salivation, dyspnoea, inco-ordination, defaecation and urination. Methionine enabled the calf to withstand doses of 0·6–0·8 mg. of Co without symptoms. In view of the generally held belief that vitamin B_{12} is produced only in certain bacteria the occurrence of B_{12} in alfalfa is of interest(498). It may well be a product of the symbiotic bacteria in the root nodules. The occurrence of B_{12} in bacteria epiphytic upon seaweeds has also been reported(499). The successful use of concentrates of so-called 'animal-protein-factor' in stimulating the growth of chicks and pigs has led to the use of sources of it for other farm animals such as calves(500,501) and lambs. The animal protein factor appears in these experiments to be a mixture of an antibiotic such as streptomycin or aureomycin and vitamin B_{12}; in one such experiment(500) B_{12} itself produced no increase of thrift. In another the growth of calves was improved by a concentrate containing aureomycin and B_{12}(501). The experiments with lambs(502,503) were unsuccessful. In adult sheep(504) aureomycin had deleterious effects on the rumen flora, and in steers(505) digestibility of crude fibre was adversely affected. Boxer & Rickards(506) have worked out a chemical method of determining B_{12} based on its cyanide content, and have incidentally shown that cyanide is a normal constituent of human and rat breath. ^{60}Co does not exchange with Co in vitamin B_{12} even after weeks of contact(507). Vitamin B_{12} forms compounds with H_2S, SO_2, Br', SO_4'', CNO', and NO_2' as well as with CN'(508). This chemical evidence makes one wonder whether it has a detoxicative role in the metabolism of herbivorous animals.

COPPER AND MOLYBDENUM

Sellers(509) reports an outbreak of swayback in 217 lambs from 159 ewes. Of these fifty-nine were unable to stand at birth and died within 48 hr. and another thirty-one died within a month with various degrees of paralysis. Blood coppers were low and *port-mortem* dissection showed demyelination and symmetrical cavitation of the cerebrum. The farm on which the cases occurred had introduced deep ploughing 3 years before, which may conceivably have affected the Cu content of the forage. In Britain the incidence of swayback in sheep and Cu cachexia in cattle does not correlate well with either the Cu or the Mo content of pastures, and Allcroft(510,511) therefore posits some other factor also in these two syndromes. This hypothesis is of interest in view of experiments by Dick(512) who found that the ratio of oaten hay to lucerne hay in the diet of

sheep altered the hepatic stores of Cu and the concentration of Mo in the blood in spite of equality of the intakes of Cu and Mo in all groups of animals. The hepatic Cu increased with increasing intake of oat hay. Dunlop(513) has shown how treatment of pregnant ewes with 200 mg. copper sulphate in solution reduced to nil from 1941 to 1948 the incidence of swayback in lambs. Swayback still persisted in untreated flocks. The disease could be prevented by copper sulphate in the remainder still to lamb from flocks showing an initial incidence. The value of Cu treatment of sheep and cows has also been reported from Cape Province in South Africa(514). Archibald(515) noted that 272 mg. Mo per day as ammonium molybdate caused a fivefold increase in the Mo in cow's milk from an average of 70 μg./l. in control cows. This was a bigger increase than could be produced with Mn, Zn, Co or Ni. The Mo in cream was doubled. Among various toxic symptoms of the administration of Na molybdate to young dairy bulls were diarrhoea, diminished growth, anaemia, graying of black hair, lameness, and degeneration of seminiferous tubules and interstitial tissue of both testes(516).

OTHER MINERALS

Bentley & Phillips(517) have reared cows from calfhood on rations poor in manganese (less than 10 p.p.m. Mn). The ration contained legume hay (poor in Mn) corn silage, corn and corn products. Growth was normal and was not stimulated by adding Mn up to 60 p.p.m. Heifers raised on this regime were slow to show oestrus and slower to conceive when bred. Manganese had no effect on vitamin A, vitamin C or carotene in the blood. The thyroid of the unborn calf gathers radio-iodine faster than that of the dam(518). This may be partly due to the excessive metabolism of young tissues and partly due to the relative quiescence of the dam's thyroid during pregnancy. A report from Japan that dithizone, which combines with zinc, produces diabetes through selective destruction of the β-cells of the pancreas has led to studies of the effect of alloxan on the metabolism of Zn. It was found that alloxan caused loss of Zn from the body. In the same paper a report is given of work by Kuhn showing the inhibition of the toxicity of alloxan by boric acid(519). Fluorine is still a hazard of grazing animals albeit in part man-made(520) due to the increasing manufacture of metallic aluminium. In a carefully planned experiment Peirce(521) has compared the effects of various intakes of fluorine from water containing 2·5, 5, 10 or 20 p.p.m. F. Mean daily F intakes were 5, 9, 18, 37 mg. in winter and 7, 13, 27 and 53 in summer. At the end of 2½ years the sheep receiving 20 p.p.m. weighed somewhat less than the others. Mottling of the incisors was noticeable at 5 p.p.m. and was marked at 20. Selective abrasion of the molars was marked at 20 and noticeable at 10 p.p.m. Fluorine caused the incisors to erupt earlier and increased the F content of teeth and bones up to twenty-fold. Hobbs *et al.*(522) in an experiment lasting 2 years have made very similar observations on cattle, but their F was added to the rations. Erosion, mottling and pitting of the teeth were produced by 40 to 50 p.p.m. F. Becker, Griffith, Hobbs & MacIntyre(523) found that symptoms of toxicity of fluorine in sheep could be alleviated by adding alum to the sheep's diet. By using the ratio of magnesium to lignin in grass Garner(524) found that 63–74% of the Mg in grass was retained by growing cattle and 65–86% by growing guinea-pigs. Lead is a nutritional hazard for farm livestock for the same reason as it is a hazard for men; it is often used as a pigment in paints. The toxicity of lead for calves and sheep has been studied by Allcroft(525,526), by Blaxter(527,528) and by Allcroft & Blaxter(529). Ingested Pb largely passes out in the faeces of sheep or rabbits. Urinary Pb reflects the intake and in the milk of the ewe Pb appears in large amounts if eaten. When the dose is large the main channel of excretion is the bile. Injected Pb is very slowly excreted(527). With increasing oral doses(528) the amount of Pb in the bones and in the kidneys increases. The amount in the kidney cortex is of special diagnostic value(525). Bovines can ingest 1–2 g. Pb per day for 2 years without ill effects(525). In young calves 200–400 mg. Pb/kg. body weight causes death in a few days. Mature sheep can withstand a slightly larger dose than this(529).

Poisoning of cattle from herbage growing on seleniferous soil has been reported from Ireland(530). The soil had 30–324 p.p.m. Se compared with only 2 p.p.m. in healthy soil nearby.

Mixed herbage contained 150–500 p.p.m. compared with 1 p.p.m. in healthy soil. 5 p.p.m. is considered dangerous. The authors compare the local geology with that of the 'alkali' lands where the same disease occurs in the U.S.A.

BRACKEN POISONING AND OTHER TOXIC SUBSTANCES

McLauchlan(531) reports twelve cases of bracken poisoning from a herd of seventy-nine cattle. Guilhon, Drieux & Joulou(532) report from France a haemorrhagic syndrome in Breton cattle believed to be due to feeding of bracken to them indoors in winter, and Jacquet(533) recommends antihaemorrhagic treatment for poisoning of cattle by bracken. Perkins(534) confirms previous workers' reports that bracken is fatal to cattle. Sheep are not so susceptible, and goats have a disinclination to eat it. He also finds that guinea-pigs and rabbits can eat it for long periods with impunity. In cattle it takes 4–8 weeks for toxic symptoms to appear and at autopsy there are internal and subcutaneous haemorrhages in the thoracic and abdominal cavities. Perkins posits a thermolabile cumulative toxin. Carpenter, Phillipson & Thomson(535) found that a sample of bracken which produced thiamine deficiency in the rat killed one of two cows which had been given 7½ lb. per day of it to eat for 55 days. Thiamine did not alleviate the symptoms. Naftalin & Cushnie(536) found changes in the bone marrow of cattle killed by bracken. Similarly, Osebold(537), by examining the blood of six cows suffering from bracken poisoning, found changes suggestive of aplastic anaemia. Moon & Raafat(538) killed a bullock and two sheep by feeding them artificially dried bracken. There was a rapid disappearance of thiamine from the urine but only terminally did pyruvic acid appear in the blood and the urine. Thiamine injections were of no value and blood prothrombin times were not abnormal, thus ruling out an induced vitamin K deficiency as a possible cause of haemorrhages. Livers of three bracken-fed sheep had 25, 120 and 210 Moore's blue units vitamin A per g. Hepatic concentrations of vitamin C were also subnormal. The same authors(539) have studied a cyanogenic principle of the bracken. They further find(540) that bracken poisoning in the sheep does not occur in the field because of the sheep's avoidance of it as a forage. Young plants are most toxic and the toxicity is destroyed by heat and extracted by treatment of the bracken with alcohol. At autospy, sheep dead of bracken poisoning have haemorrhages all along the gut.

Blakeley & Coop(541) have found that HCN in the rumen is detoxicated mainly as thiocyanate and is thereafter excreted in the urine for several days. Serum CNS^- became maximal 15 min. after dosing with HCN. *In vitro* ruminal fluid increased the rate of disappearnce of HCN but boiled fluid did not. Added cystine or thiosulphate hastened the disappearance presumably by acting as donors of sulphur. The same workers(542) have measured the lethal dosage of HCN from the cyanogenic glucoside from white clover (lotaustralin) for sheep. Hornby & Robson(543) fed sheep on a cyanogenic grass (*Cynodon plectostashyum*). Three sheep were given the *Cynodon* and three were not. All were given 3 mg. Na CN per lb. and showed no signs of poisoning. Only with 5 mg. as a drench did poisoning appear and the effect was greater in the sheep on the grass not containing the cyanogenic principle. The authors conclude that, in spite of its HCN content the *Cynodon* is a good fodder. These experiments are reminiscent of those done earlier at Onderstepoort in South Africa by Clark & Quin(544). The Western Australian workers have continued their studies of the oestrogenic effects of *Trifolium subterraneum* which they originally discovered (545). Green clover was potent, dry clover resulting from the onset of summer was not. In another study by Underwood & Shier(546) 5-tooth, 4-tooth and 2-tooth ewes were taken from subterranean clover land to normal herbage. Breeding performances revealed that the damage was irreversible and was the more severe the longer the exposure had been. The effects varied from abortion to infertility. Alexander & Rossiter(547) found that clover from plots not recently fertilized was more potent than that from plots receiving superphosphate. Beck & Braden(548), who are attempting to isolate the toxic principle, have evidence that it is not one of the common steroid oestrogens. Dohan, Richardson, Stribley & Gyorgy(549) found oestrogen in the phenolic fraction of methanolic extracts of clover and spring rye grass but not in orchard grass, spring wheat, blue grass, alfalfa or autumn rye grass.

Depredations of insects are so widespread and costly that insecticides are very frequently employed to combat infestation. It is therefore important to know how toxic they may be to farm animals and man. Up to 40 p.p.m. of parathion in roughage failed to produce any detectable amount of parathion in the milk of cows. Flies did not die when allowed to drink the milk. One part per million in the roughage would be sufficient for insect control(550,551). Alfalfa hay containing up to 42 p.p.m. DDT produced no deleterious effects in lambs eating the hay with grain(552). In a similar experiment with calves(553) hay with 106 p.p.m. DDT was consumed without ill effects in spite of the storage of as much as 0·345 p.p.m. in the body fat. However, in another experiment with bull calves(554) DDT caused skin wrinkling and thickening, profuse scaliness, lacrymation, nasal catarrh and tremors. DDT in forage is passed to the cow's milk faster than is DDT crystalline or in solution in oil(555). Brito-Babapulle(556) used riboflavine as an antidote to DDT.

In an experiment on a Holstein-Friesian steer 30 g. oxalic acid administered every day in a gelatine capsule for 45 days produced inflamed ureters and kidney damage but no calculus(557). Sulphathalidine(558) reduced the output of thiamine in the faeces of heifers without affecting the urinary thiamine. Other B complex factors in urine and faeces were unaffected. An attempt to identify the icterogenic factor of facial eczema in South African sheep, with nitrosated chlorophyll has not succeeded(559). Laurence *et al.*(560) in South Africa have investigated the severity of worm infestation in sheep on two different planes of nutrition. A mass mixed infestation of *Haemonchus contortus* and *Oesophagostomum columbianium* was used. In lambs 7 to 8 months old such an infestation was rapidly fatal, irrespective of the diet. In lambs 10–11 months old a similar infestation led to chronic verminosis, but the lambs receiving the extra 300 g. maize per day grew faster, ate more hay and maintained their blood haemoglobin, fleece weight and fleece fibre diameter better than the non-supplemented but infected animals.

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